JC10 Rec'd PCT/PTO 2 8 SEP 2001

FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **14114.0353U2**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR: 09/937862

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/07828	**24 March 2000**	**31 March 1999**

TITLE OF INVENTION
TYPING OF HUMAN ENTEROVIRUSES

APPLICANT(S) FOR DO/EO/US
OBERSTE ***et al.***

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail #EL491885455US
20. ☒ Other items or information:

SEQUENCE LISTING DISKETTE; SEQUENCE LISTING IN WRITTEN FORM (38 PAGES); A COPY OF TWO (2) REQUESTS FOR RECORDING OF A CHANGE UNDER PCT RULE 92BIS; RETURN POSTCARD

EL491885455US

PCTUS1/REV03

410 Rec'd PCT/PTO 28 SEP 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/937862	INTERNATIONAL APPLICATION NO. PCT/US00/07828	ATTORNEY'S DOCKET NUMBER 14114.0353U2

21. The following fees are submitted:.

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,000.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO **$860.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	46 - 20 =	26	x $18.00	**$468.00**	
Independent claims	7 - 3 =	4	x $80.00	**$320.00**	
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,648.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable).** ☐				**$0.00**	
SUBTOTAL =				**$1,648.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,648.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,648.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$1,648.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees. A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **14-0629** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

MILLER, Mary L.
NEEDLE & ROSENBERG, P.C.
127 Peachtree Street, N.E.
Suite 1200
Atlanta, Georgia 30303-1811

Mary L. Miller
SIGNATURE

MARY L. MILLER
NAME

39,303
REGISTRATION NUMBER

September 28, 2001
DATE

DOCKET NUMBER 14114.0353U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
OBERSTE *et al.*	)	Group Art: Unassigned
Confirmation No. 8841	)	
Serial No. 09/937,862	)	
Filed: September 28, 2001	)	Examiner: Unassigned
For: **"TYPING OF HUMAN ENTEROVIRUSES"**	)	

RESPONSE TO NOTIFICATION OF MISSING REQUIREMENTS

Attn: Ms. Vonda M. Wallace
Commissioner for Patents
BOX PCT
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

February 14, 2002

Sir:

In response to the December 14, 2001 Notification of Missing Requirements Under 35 U.S.C. §371 which has been issued in the above-identified patent application, enclosed are

1. A substitute Sequence Listing diskette;
2. a substitute Sequence Listing in paper form with corrections as required in Notice (39 Pages);
3. a copy of the Notification of Missing Requirements Under 35 U.S.C. 371 in the United States Designated/Elected Office (DO/EO/US); and
4. a return postcard.

The enclosed diskette containing the Sequence Listing for this application in computer readable form (CRF) is submitted in compliance with 37 C.F.R. §§ 1.821-1.825. Applicants hereby certify that the information in both the computer readable form included herewith and the paper copy of the substitute Sequence Listing as included herewith is the same and includes no new matter.

Applicants hereby request amendment to the specification by replacing the Sequence Listing filed with the application on September 28, 2001, with the enclosed, substitute Sequence Listing. Entry of the substitute Sequence Listing is respectfully requested.

No fee is believed due. However, the Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account No. 14-0629.

Respectfully submitted,

NEEDLE & ROSENBERG, P.C.

Mary L. Miller

Mary L. Miller
Registration No. 39,303

Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811
(404) 688-0770

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mailing No. EL491885680US in an envelope addressed to: Attn: Ms. Vonda M. Wallace, Commissioner for Patents, BOX PCT, Washington, D.C. 20231, on the date shown below.

Erick Calderon

Erick Calderon

2/14/02

Date

ENTERED PCT09

P,6

```
RAW SEQUENCE LISTING                                DATE: 03/07/2002
PATENT APPLICATION:  US/09/937,862A                 TIME: 15:23:26

Input Set : A:\14114.0353U2.TXT
Output Set: N:\CRF3\03072002\I937862A.raw

      4 <110> APPLICANT: Oberste, M. Steven
      5       Maher, Kaija
      6       Kilpatrick, David R.
      7       Pallansch, Mark A.
     10 <120> TITLE OF INVENTION: TYPING OF HUMAN NON-POLIO ENTEROVIRUSES
     13 <130> FILE REFERENCE: 14114.0353U2
     15 <140> CURRENT APPLICATION NUMBER: 09/937,862A
C--> 16 <141> CURRENT FILING DATE: 2002-02-14
     18 <150> PRIOR APPLICATION NUMBER: PCT/US00/07828
     19 <151> PRIOR FILING DATE: 2000-03-24
     21 <150> PRIOR APPLICATION NUMBER: 60/127,464
     22 <151> PRIOR FILING DATE: 1999-03-31
     24 <160> NUMBER OF SEQ ID NOS: 86
     26 <170> SOFTWARE: FastSEQ for Windows Version 4.0
     28 <210> SEQ ID NO: 1
     29 <211> LENGTH: 20
     30 <212> TYPE: DNA
     31 <213> ORGANISM: Artificial Sequence
     33 <220> FEATURE:
     34 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     35       synthetic construct
     38 <400> SEQUENCE: 1
     39 gcrtgcaatg ayttctcwgt                                                20
     41 <210> SEQ ID NO: 2
     42 <211> LENGTH: 18
     43 <212> TYPE: DNA
     44 <213> ORGANISM: Artificial Sequence
     46 <220> FEATURE:
     47 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     48       synthetic construct
W--> 51 <221> NAME/KEY: misc_feature
     52 <222> LOCATION: (1)...(18)
     53 <223> OTHER INFORMATION: n = a, t, c or g
W--> 55 <400> 2
W--> 56 ngcnccdgat tgntgscc                                                  18
     58 <210> SEQ ID NO: 3
     59 <211> LENGTH: 20
     60 <212> TYPE: DNA
     61 <213> ORGANISM: Artificial Sequence
     63 <220> FEATURE:
     64 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     65       synthetic construct
W--> 68 <221> NAME/KEY: misc_feature
```

```
                    RAW SEQUENCE LISTING                        DATE: 03/07/2002
                    PATENT APPLICATION:  US/09/937,862A          TIME: 15:23:26

                    Input Set : A:\14114.0353U2.TXT
                    Output Set: N:\CRF3\03072002\I937862A.raw

     69 <222> LOCATION: (1)...(20)
     70 <223> OTHER INFORMATION: n = a, t, c or g
W--> 72 <400> 3
W--> 73 gcnccngayt gntgnccraa                                                 20
     75 <210> SEQ ID NO: 4
     76 <211> LENGTH: 20
     77 <212> TYPE: DNA
     78 <213> ORGANISM: Artificial Sequence
     80 <220> FEATURE:
     81 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     82       synthetic construct
W--> 85 <221> NAME/KEY: misc_feature
     86 <222> LOCATION: (1)...(20)
     87 <223> OTHER INFORMATION: n = a, t, c or g
W--> 89 <400> 4
W--> 90 atgtaygtnc cnccnggngg                                                 20
     92 <210> SEQ ID NO: 5
     93 <211> LENGTH: 20
     94 <212> TYPE: DNA
     95 <213> ORGANISM: Artificial Sequence
     97 <220> FEATURE:
     98 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     99       synthetic construct
W--> 102 <221> NAME/KEY: misc_feature
     103 <222> LOCATION: (1)...(20)
     104 <223> OTHER INFORMATION: n = a, t, c or g
W--> 106 <400> 5
W--> 107 ggngcrttnc cytcngtcca                                                20
     109 <210> SEQ ID NO: 6
     110 <211> LENGTH: 20
     111 <212> TYPE: DNA
     112 <213> ORGANISM: Artificial Sequence
     114 <220> FEATURE:
     115 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     116       synthetic construct
W--> 119 <221> NAME/KEY: misc_feature
     120 <222> LOCATION: (1)...(20)
     121 <223> OTHER INFORMATION: n = a, t, c or g
W--> 123 <400> 6
W--> 124 acrtgncnng tytgcatngt                                                20
     126 <210> SEQ ID NO: 7
     127 <211> LENGTH: 18
     128 <212> TYPE: DNA
     129 <213> ORGANISM: Artificial Sequence
     131 <220> FEATURE:
     132 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
     133       synthetic construct
W--> 136 <221> NAME/KEY: misc_feature
     137 <222> LOCATION: (1)...(18)
```

RAW SEQUENCE LISTING DATE: 03/07/2002
PATENT APPLICATION: **US/09/937,862A** TIME: 15:23:26

Input Set : **A:\14114.0353U2.TXT**
Output Set: **N:\CRF3\03072002\I937862A.raw**

138 <223> OTHER INFORMATION: n = a, t, c or g
W--> 140 <400> 7
W--> 141 awnttytayg ayggntgg **18**
143 <210> SEQ ID NO: 8
144 <211> LENGTH: 20
145 <212> TYPE: DNA
146 <213> ORGANISM: Artificial Sequence
148 <220> FEATURE:
149 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
150 synthetic construct
W--> 153 <221> NAME/KEY: misc_feature
154 <222> LOCATION: (1)...(20)
155 <223> OTHER INFORMATION: n = a, t, c or g
W--> 157 <400> 8
W--> 158 tananngtnc ccatrttrtt **20**
160 <210> SEQ ID NO: 9
161 <211> LENGTH: 20
162 <212> TYPE: DNA
163 <213> ORGANISM: Artificial Sequence
165 <220> FEATURE:
166 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
167 synthetic construct
W--> 170 <221> NAME/KEY: misc_feature
171 <222> LOCATION: (1)...(20)
172 <223> OTHER INFORMATION: n = a, t, c or g
W--> 174 <400> 9
W--> 175 atgtayrtnc cnmcnggngc **20**
177 <210> SEQ ID NO: 10
178 <211> LENGTH: 20
179 <212> TYPE: DNA
180 <213> ORGANISM: Artificial Sequence
182 <220> FEATURE:
183 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
184 synthetic construct
W--> 187 <221> NAME/KEY: misc_feature
188 <222> LOCATION: (1)...(20)
189 <223> OTHER INFORMATION: n = a, t, c or g
W--> 191 <400> 10
W--> 192 ggnggnggrt cngtnakytt **20**
194 <210> SEQ ID NO: 11
195 <211> LENGTH: 20
196 <212> TYPE: DNA
197 <213> ORGANISM: Artificial Sequence
199 <220> FEATURE:
200 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
201 synthetic construct
W--> 204 <221> NAME/KEY: misc_feature
205 <222> LOCATION: (1)...(20)
206 <223> OTHER INFORMATION: n = a, t, c or g

RAW SEQUENCE LISTING DATE: 03/07/2002
PATENT APPLICATION: US/09/937,862A TIME: 15:23:26

Input Set : A:\14114.0353U2.TXT
Output Set: N:\CRF3\03072002\I937862A.raw

W--> 208 <400> 11
W--> 209 gangaraayc tnatngarac 20
211 <210> SEQ ID NO: 12
212 <211> LENGTH: 19
213 <212> TYPE: DNA
214 <213> ORGANISM: Artificial Sequence
216 <220> FEATURE:
217 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
218 synthetic construct
W--> 221 <221> NAME/KEY: misc_feature
222 <222> LOCATION: (1)...(19)
223 <223> OTHER INFORMATION: n = a, t, c or g
W--> 225 <400> 12
W--> 226 cccatnakrt cnatrtccc 19
228 <210> SEQ ID NO: 13
229 <211> LENGTH: 20
230 <212> TYPE: DNA
231 <213> ORGANISM: Artificial Sequence
233 <220> FEATURE:
234 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
235 synthetic construct
W--> 238 <221> NAME/KEY: misc_feature
239 <222> LOCATION: (1)...(20)
240 <223> OTHER INFORMATION: n = a, t, c or g
W--> 242 <400> 13
W--> 243 gtrctyacna nnagrtcyct 20
245 <210> SEQ ID NO: 14
246 <211> LENGTH: 19
247 <212> TYPE: DNA
248 <213> ORGANISM: Artificial Sequence
250 <220> FEATURE:
251 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
252 synthetic construct
W--> 255 <221> NAME/KEY: misc_feature
256 <222> LOCATION: (1)...(19)
257 <223> OTHER INFORMATION: n = a, t, c or g
W--> 259 <400> 14
260 tsaarytgtg caargacac 19
262 <210> SEQ ID NO: 15
263 <211> LENGTH: 18
264 <212> TYPE: DNA
265 <213> ORGANISM: Artificial Sequence
267 <220> FEATURE:
268 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
269 synthetic construct
W--> 272 <221> NAME/KEY: misc_feature
273 <222> LOCATION: (1)...(18)
274 <223> OTHER INFORMATION: n = a, t, c or g
W--> 276 <400> 15

RAW SEQUENCE LISTING DATE: 03/07/2002
PATENT APPLICATION: US/09/937,862A TIME: 15:23:26

Input Set : A:\14114.0353U2.TXT
Output Set: N:\CRF3\03072002\I937862A.raw

277 stgyccagat ttcagtgt 18
279 <210> SEQ ID NO: 16
280 <211> LENGTH: 20
281 <212> TYPE: DNA
282 <213> ORGANISM: Artificial Sequence
284 <220> FEATURE:
285 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
286 synthetic construct
W--> 289 **<221> NAME/KEY: misc_feature**
290 <222> LOCATION: (1)...(20)
291 <223> OTHER INFORMATION: n = a, t, c or g
W--> 293 **<400> 16**
W--> 294 **ggnacncayr tnathtggga** 20
296 <210> SEQ ID NO: 17
297 <211> LENGTH: 20
298 <212> TYPE: DNA
299 <213> ORGANISM: Artificial Sequence
301 <220> FEATURE:
302 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
303 synthetic construct
W--> 306 **<221> NAME/KEY: misc_feature**
307 <222> LOCATION: (1)...(20)
308 <223> OTHER INFORMATION: n = a, t, c or g
W--> 310 **<400> 17**
W--> 311 **gccntrttnt grtgnccraa** 20
313 <210> SEQ ID NO: 18
314 <211> LENGTH: 20
315 <212> TYPE: DNA
316 <213> ORGANISM: Artificial Sequence
318 <220> FEATURE:
319 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
320 synthetic construct
W--> 323 **<221> NAME/KEY: misc_feature**
324 <222> LOCATION: (1)...(20)
325 <223> OTHER INFORMATION: n = a, t, c or g
W--> 327 **<400> 18**
W--> 328 **ggnacncayr tnrtntggga** 20
330 <210> SEQ ID NO: 19
331 <211> LENGTH: 20
332 <212> TYPE: DNA
333 <213> ORGANISM: Artificial Sequence
335 <220> FEATURE:
336 <223> OTHER INFORMATION: Description of Artificial Sequence; Note =
337 synthetic construct
W--> 340 **<221> NAME/KEY: misc_feature**
341 <222> LOCATION: (1)...(20)
342 <223> OTHER INFORMATION: n = a, t, c or g
W--> 344 **<400> 19**
W--> 345 **acngcngyng aracnggnca** 20

RAW SEQUENCE LISTING ERROR SUMMARY   DATE: 03/07/2002
PATENT APPLICATION: US/09/937,862A   TIME: 15:23:27

Input Set : A:\14114.0353U2.TXT
Output Set: N:\CRF3\03072002\I937862A.raw

Please Note:
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:2; N Pos. 1,4,13
Seq#:3; N Pos. 3,6,12,15
Seq#:4; N Pos. 9,12,15,18
Seq#:5; N Pos. 3,9,15
Seq#:6; N Pos. 6,8,9,18
Seq#:7; N Pos. 3,15
Seq#:8; N Pos. 3,5,6,9
Seq#:9; N Pos. 9,12,15,18
Seq#:10; N Pos. 3,6,12,15
Seq#:11; N Pos. 3,12,15
Seq#:12; N Pos. 6,12
Seq#:13; N Pos. 9,11,12
Seq#:16; N Pos. 3,6,12
Seq#:17; N Pos. 4,9,15
Seq#:18; N Pos. 3,6,12,15
Seq#:19; N Pos. 3,6,9,15,18
Seq#:20; N Pos. 3,6,9,15,18
Seq#:21; N Pos. 6,9,15,18
Seq#:22; N Pos. 2,5,8,11
Seq#:81; Xaa Pos. 3,5
Seq#:82; Xaa Pos. 3
Seq#:83; Xaa Pos. 3
Seq#:84; Xaa Pos. 7
Seq#:86; Xaa Pos. 2,3,7

```
VERIFICATION SUMMARY                              DATE: 03/07/2002
PATENT APPLICATION:  US/09/937,862A                TIME: 15:23:27

Input Set : A:\14114.0353U2.TXT
Output Set: N:\CRF3\03072002\I937862A.raw
```

```
L:16 M:271 C: Current Filing Date differs, Replaced Current Filing Date
L:51 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:55 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:2
L:56 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:2 after pos.:0
L:68 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:72 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:3
L:73 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:0
L:85 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:89 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:4
L:90 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4 after pos.:0
L:102 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:106 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:5
L:107 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:5 after pos.:0
L:119 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:123 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:6
L:124 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:6 after pos.:0
L:136 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:140 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:7
L:141 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:7 after pos.:0
L:153 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:157 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:8
L:158 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:8 after pos.:0
L:170 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:174 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:9
L:175 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:9 after pos.:0
L:187 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:191 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:10
L:192 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:10 after pos.:0
L:204 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:208 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:11
L:209 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:11 after pos.:0
L:221 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:225 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:12
L:226 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:12 after pos.:0
L:238 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:242 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:13
L:243 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:13 after pos.:0
L:255 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:259 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:14
L:272 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:276 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:15
L:289 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:293 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:16
L:294 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:16 after pos.:0
L:306 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:310 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:17
L:311 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:17 after pos.:0
L:323 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
```

VERIFICATION SUMMARY DATE: 03/07/2002
PATENT APPLICATION: **US/09/937,862A** TIME: 15:23:27

Input Set : **A:\14114.0353U2.TXT**
Output Set: **N:\CRF3\03072002\I937862A.raw**

```
L:327 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:18
L:328 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:18 after pos.:0
L:340 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:344 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:19
L:345 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:19 after pos.:0
L:357 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:361 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:20
L:362 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:20 after pos.:0
L:374 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:378 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:21
L:379 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:21 after pos.:0
L:391 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:395 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:22
L:396 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:22 after pos.:0
L:1972 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:1976 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:81
L:1977 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:81 after pos.:0
L:1990 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:1994 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:82
L:1995 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:82 after pos.:0
L:2008 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:2012 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:83
L:2013 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:83 after pos.:0
L:2026 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:2030 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:84
L:2031 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:84 after pos.:0
L:2058 M:281 W: Numeric Fields not Ordered, <221> Sort in ascending order!
L:2062 M:258 W: Mandatory Feature missing, <220> not found for SEQ ID#:86
L:2063 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:86 after pos.:0
```

#4

SEQUENCE LISTING

<110> Oberste, M. Steven
Maher, Kaija
Kilpatrick, David R.
Pallansch, Mark A.

<120> TYPING OF HUMAN NON-POLIO ENTEROVIRUSES

<130> 14114.0353U2

<140> 09/937,862
<141> 2001-09-28

<150> PCT/US00/07828
<151> 2000-03-24

<150> 60/127,464
<151> 1999-03-31

<160> 86

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 1
gcrtgcaatg ayttctcwgt 20

<210> 2
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(18)
<223> n = a, t, c or g

<400> 2
ngcnccdgat tgntgscc 18

<210> 3
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 3
gcnccngayt gntgnccraa 20

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 4
atgtaygtnc cnccnggngg 20

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 5
ggngcrttnc cytcngtcca 20

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 6
acrtgncnng tytgcatngt 20

<210> 7
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(18)
<223> n = a, t, c or g

<400> 7
awnttytayg ayggntgg 18

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 8
tananngtnc ccatrttrtt 20

<210> 9
<211> 20
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 9
atgtayrtnc cnmcnggngc 20

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 10
ggnggnggrt cngtnakytt 20

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 11
gangaraayc tnatngarac 20

<210> 12
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(19)
<223> n = a, t, c or g

<400> 12
cccatnakrt cnatrtccc 19

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 13
gtrctyacna nnagrtcyct 20

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(19)
<223> n = a, t, c or g

<400> 14
tsaarytgtg caargacac 19

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(18)
<223> n = a, t, c or g

<400> 15
stgyccagat ttcagtgt 18

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 16
ggnacncayr tnathtggga 20

<210> 17
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 17
gccntrttnt grtgnccraa 20

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 18
ggnacncayr tnrtntggga 20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 19
acngcngyng aracnggnca 20

<210> 20
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(19)
<223> n = a, t, c or g

<400> 20
acngcngtng aracnggng 19

<210> 21
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(20)
<223> n = a, t, c or g

<400> 21
cargcngcng aracnggngc 20

<210> 22
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> misc_feature
<222> (1)...(19)
<223> n = a, t, c or g

<400> 22
cnccnggngg nayrwacat 19

<210> 23
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 23
ggattgggcg attctattga ggctgccatt gacagcatca cacaaaatgc actaaccact 60
gtacaaaata caacacaatc aggacctact cattcaaaag aagttccagc attaacagca 120
gtggaaacag gtgctactag tcaagtagaa ccaggtgact tgattgaaac cagacatgtt 180
ataaacatga gacaaagatc tgaagcatct atcgaatctt tctttggccg atccgcatgt 240
gttgcgatac ttggtttgtc aaacgccaaa ccaactgaca caaacaccaa acaattgttc 300
aaaacatgga gaatatcata tttagaaact caccaactca gaagaaaact tgagttcttt 360
acgtactcaa ggtttgattt ggaaatgacc atagtaatta cagagagggt tttcaatgca 420
gtcaatgtcc cattgcgcaa ttatgtgtac caaataatgt acgttccccc aggtgctcca 480
gaaccacaat catgggatga ttacacgtgg caatcttcta ccaacccatc aatattctac 540
accactggaa atgctcctcc cagagtgtca attccatttg ttggaatagg gtctgcatat 600
tcacactttt atgatggttt ctcacagatt cctcttgact caatcagtgc tggagcaagt 660
aataagtatg gttacacttc aatcaatgac tttggtaccc tggcaattag aatagtaaat 720
gaatatgacc cagtgcaagt ggatgcaaag gcccgagtgt atattaaacc caaacatgtt 780
cgcatgtggt gccccagacc accacgggcc atgccttaca agaatagcac agtggatttc 840
gacccatcag caactgtaat gacccaagtc gcagacatca ggacgtat 888

<210> 24
<211> 882
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 24
ggagatccag tggaagactt aatcgccaat acagttgcta ggactctaga gagaataacc 60
tctccaactc ataatacaac ggcaggcaac accaccgtta gcgagcacag catcggtacc 120
ggttcagtgc ctgcgttgca agctgctgag actggggctt cgtctaacac cacagatgag 180
agtatgatag aaacacggtg tgttgtcaat aggaatggag tgattgagac tagcatcaac 240
catttcttct cccgagcggg gcttgtggga gtgctgaaca tacttgatgg aggcacctca 300
aaaggctttg aagtttggga tatagacatc atgggctttg ttcagcttcg cagaaagcta 360
gagatgttca cctacatgcg gttcaacgct gaattcacct ttgtcgcgac tttgagtgac 420
ggaacaactc cccatataat gttgcaatac atgtatgtgc cccctggagc tcccaaacct 480
caggaaagag attcattcca atggcagact gcaaccaacc catccgtgtt tgcgaaaatg 540
agtgaccctc ctccgcaagt ttcagtacct ttcatgtctc ctgctagcgc ctaccagtgg 600
ttttatgatg ggtacccaac atttgatgat agaccacaga cctctaatcg tccctacgga 660
caatgcccca ataacatgtt gggcacattc gcggtgcgca ttgttagcaa gacgcctgcg 720
gagagagact tgcgcgtccg tgtttacatg aaactgaagc atgtgcgagc atgggtaccg 780
cgacccataa ggtcacagcc ttacgtcttg aagaactacc ccaactatga tggaacccaa 840
atcgtgccca gtgccaaaga tcgagaagac ataaagaaca ca 882

<210> 25
<211> 915
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 25
ggtgatgcaa tcgctgatgc tatacaaaac acagttacat ctactataca gagagtcaca 60
accaacactg ttgggcaaga tgcaacagct gctaacacag cacccagctc tcatagtttg 120
aacactggcc tagtccccgc gcttcaagct gctgagacag gagcttcatc cacagccacg 180
gatgggaatt tgattgagac tagatgtgtt gtaaactcca atggtacacg tgaaacccac 240
attgagcatt tcttctctag gtcagggctg gtgggagtta tggaggtaga tgatacgggt 300
actagtggca agggattctc aaactgggac attgacatca tggcgtttgt gcaactgcgc 360
cgtaaactcg aggcatttac atatatgcgg ttcgacgcag agtttacctt tgtcaccaat 420
ttggagaacg ggctcacgaa taatagtgtg atacagtaca tgtatgtacc acctggagcg 480
cctaaacccg atgcccggga atcattccag tggcaaactg caaccaatcc gtcagtcttt 540
caaaaaatgg acagtccgcc acctcaagtt tcagtaccct tcatgtcacc agccagtgcc 600
tatcaatggt tctatgacgg ttaccccacc tttgggcccc actcggagac atctaatcta 660
tcttacgggc aatgtcccaa taatatgctg ggaacattct cggccagggt tgttagcaag 720
caaatcacca atcagaaatt ccagatccgt atttatctac ggctgaagag ggtgagggcg 780
tggatcccca gacctttgag atcgcagccg tacatttaca gaaactaccc cacctatggt 840
actaccatcc aatacctggc caaagatagg cgcaagatca ctgaaactga ttataatgct 900
gaacagcgca cgcat 915

<210> 26
<211> 885
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 26
ggcagaccaa ttgcagatat aatagaagga gcagtagctc aaactaccac cagagcacta 60
agtggaccaa ttcagccagt gacagcggcc aacacctctc ccagttcaca tcggcttggt 120
acggggcaag tgccagcttt gcaagcagca gaaacgggag ccacctcgaa tgcgaccgac 180
gagagtttga ttgaaaccag gtgtgtggtc aacagacatg gagtcatgga aactagcatt 240
gaacacttct tttcacgctc aggcttggca ggaattttga taattgagga ctccggtact 300
tccacgaaag gctacgccac ttgggaaatc gatgttatgg gatttgtcca gctgaggcgt 360
aaactagaga tgttcacata catgcgattt gatgcagagt tcacctttat cacagcagaa 420
aggaatggca acaccagccc aatacccatc cagtacatgt atgtcccacc cggagcccca 480
gtccctactg gtagggagac attccaatgg caaacagcga ccaatccatc cgtgatctca 540
aagatgactg atccaccagc ccaggtgtct gtaccattta tgagcccagc cagtacttat 600
caatggttct acgatggcta ccccacgttc ggagaagttc cagtgactac gaacttgaac 660
tatggacagt gcccaaacaa caaaatgggc actttctgca tccgcatggt ctcaggtgta 720
tctacaggca aggacgtcac tgtgcgcatt ttcatgaagt tgaagcatgt gcgcgcctgg 780
gtgccaaggc ccatcaggag ccagccttac ttgttaaaga attatcccaa ctttgacaag 840
tcaaatattg tagacgcatc atcgaacagg acatatacca ccact 885

<210> 27
<211> 915
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 27
aatgacccca tttcaaatgc aatagaaaat gctgtgagca cactcgctga caccacgata 60
tcacgtgtta cagcggccaa cactgctgct agctcccatt cccttggtac tggacgcgtg 120
ccggcgttgc aggctgcgga gacaggggca agttccaacg ctagcgatga gaacctgatt 180
gaaactcgtt gtgtgatgaa tagaaatgga gttaacgaag caagtgtaga acacttctac 240
tcccgtgcag ggctagtagg agttgtggag gtgaaagact caggcactag tcaggacggg 300
tacacggtgt ggcccataga tgtgatgggc tttgtgcaac agcggcgcaa gttagagcta 360
tctacttaca tgcgctttga cgctgaattt acctttgtgt ccaatctcaa tgacagcaca 420
acacccggca tgctattgca gtacatgtac gtgccgccgg gtgcgcccaa accagacggt 480
aggaagtcat atcaatggca aacagccacc aacccttcaa tattcgcaaa gttgagtgac 540
ccaccgcccc aagtgtctgt cccattcatg tcaccggcgt cagcctacca gtggttctac 600
gatggttacc ccacgtttgg cgaacacaag caagctacta atttacaata cggtcagtgc 660
cctaacaaca tgatggggca ttttgctatt cggacagtta gtgaatccac caccgggaaa 720
aatgtccatg tccgggtgta catgagaatt aagcacgtaa gagcatgggt gcccagacct 780
ttcagatccc aagcttacat ggtcaaaaac tacccgacat acagccaaac aatatccaat 840
actgcagccg atcgtgcgag cataaccact acggactatg agggtggcgt accagcaaac 900
ccgcagagaa ctttt 915

<210> 28
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 28

```
ggagacgaaa tactcgacct aatcgagagt gctgtacaga ataccactaa agccattacc      60
agctcaatcg acaccaaaac tggtgctaac actcaagcta gccaacatcg tataggcttg     120
ggggaggttc ccgctcttca agctgctgag acaggatcgt cttcgctcgt ttcggacaag     180
aacatgatag aaacaaggtg tgtcgtaaac aaacacagca cagaggaaac cagcattaca     240
aacttctact ccagggcggg cctagtgggg gttgtgaaca tgccagtaca aggaaccagc     300
aacacaaagg gtttcgcaaa gtgggggata gatataatgg gctttgtgca gatgaggcgc     360
aaacttgagc tcatgacata catgagattc tccgccgagt ttacgttcgt acccagcact     420
cctgggggag agactactaa ccttatactg caatacatgt atgcacctcc cggagctccg     480
ctgccaacca ggcgggattc atacgaatgg caaacatcca ctaacccctc tattatcagc     540
aagatggcgg acccacccgc tcaggtatcg gttccattcc tttctcctgc atcagcatat     600
cagtggttct atgatggcta ccccacattt gggaaacacc caatagatca ggacttccaa     660
tatggcatgt gcccaaacaa catgatgggc acattctgtg tgcgcatgat cggtgggggc     720
aaaccgaccc aatcagttac catacgtata tacatgagat taaagcatat ccgtgcatgg     780
gtgccccggc cactgaggag tcagaattac actatgagga attacccgaa ctacaacggg     840
ggcgcaataa aatgtacatc aaaaagcaga gctaccataa caacctta                 888
```

<210> 29
<211> 882
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 29

```
ggagattcca ttgaagacat aataagcaac actgtcaccc gtacactgca acaaatcagt      60
gccccatcac acgacactac agcagccaac acctcagtga gtaatcataa aattggtacg     120
ggggatgtcc cagctctcca agctgcagag actggcgcta cttccaatgc ctcagacgag     180
aacatgattg agacacgatg tgtgttaaat cgcaatgggg ttgtggaaac tagtttggac     240
catttctttt caagagcagg ccttgtggga gtgatcaatg tgcaagatgg cggcactcag     300
aagggtttg aagtgtggga catagatgtc atggggtttg ttcaactcag gaggaagttg      360
gagatgttca cgtacatgag gttcaacgcc gagttcacat tcgtatccac actcgcggat     420
ggcacaactc ccagagtgat gttgcagtac atgtacgttc cacctggtgc ccccaaacct     480
caggagagag attcgtttca gtggcaaact gcaaccaacc catcagtatt ttgcaaaatg     540
agtgaccctc ctccacaggt ttccgttcct ttcatgtcac cagctagtgc ctaccaatgg     600
ttctacgatg ggtacccaac attcgatgat cgaccggcca cctcaaacca cccgtacggt     660
cagtgcccca ataacatgat gggcacattc gcagtgcggt ttgtcagcaa gaccccagcc     720
acacgggatc tgcgtgtcag agtgtacatg cgcctgaaac acgtgcgcgc atgggtaccg     780
agacctatcc gatctcaacc ctatatttg aaaaactacc caaattatga tggcacaaag      840
ataacgtcga catctaagga taggcaaagc atcaaaacaa ca                       882
```

<210> 30
<211> 894
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 30
ggcgaccccg tggaggacat catccacgac gctttgagca gcactgtgcg gcgggccata 60
actagtggtc aagatgtcaa cacagcggcc ggtaccgctc ctagctctca caggttggag 120
actggtcgtg ttcccgccct acaagcagca gaaactggag ccacttctaa cgctacagat 180
gagaacatga tagaaacgcg gtgtgtcatg aacagaaatg gagtgttgga ggcgactata 240
agtcatttct tctcacgctc aggtttggtg ggtgttgtca atctaactga cggaggcacc 300
gatacaacgg gatatgcagt gtgggacatt gacatcatgg gttttgtgca actgcggcgg 360
aaatgtgaga tgttcacata catgagattc aacgctgagt tcacattcgt cactacaaca 420
gaaaatggcg aggcaaggcc atttatgtta cagtatatgt atgtacctcc aggtgcccct 480
aagccaacgg gtagagatgc ttttcagtgg caaacagcga caaatccatc cgttttcgtt 540
aagctcacag atccacctgc tcaggtatca gtccccttca tgtcacctgc tagtgcctac 600
caatggttct atgacgggta tccaacattt ggacaacacc cggaaacatc taatacaaca 660
tatggacagt gccctaacaa catgatgggg acctttgctg tgagagtagt gagtagagtg 720
gctagccagc tcaaactaca gacacgagtg tatatgaagc ttaagcatgt gagagcatgg 780
atccctaggc caataagatc ccagccttac ctcctaaaga attttccaaa ttatgatagt 840
agtaagatca catacagcgc aagagatcgt gccagcataa aacaagctaa tatg 894

<210> 31
<211> 912
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 31
gggccaatag aagaaatcat ctcaactgtt gccagtaacg cgttggcgct cagtcaaccc 60
aagccagtgg acaactctgt acaaaacacc caacaaagtg ctccagtgca tagccaggag 120
gtgccagcat tgaccgcagt ggagacaggg gcgacaagtg atgtggttcc atctgaccta 180
attcagacta gacacgtatt gaatgttaaa tccaggtctg aatccaccat cgagtcattt 240
tttgcaagag ctgcatgtgt aaccattatg caggtggaca atttcaacgc aacctctgtg 300
gaagacaaaa gaaagttgtt tgctaaatgg gcaatcacct acactgatac cgtccagctg 360
agacggaaat tagagttttt cacttattct agatttgact tagagatgac ttttgtgcta 420
actgagagat actactccca aagctcaggg catgctagat ctcaggtgta ccaaattatg 480
tatgttccac caggggcacc cacgcctagt gcatgggacg actacacatg gcaaacatcc 540
tccaacccat ccattttctt taccaccggc aatgcaccac cgcgcatttc aattccattt 600
gttggaatcg ccaatgcata ctcacacttt tatgatggct ttagtagagt acctttggag 660
ggagaaacaa cagacacagg agacgcttac tacggctca cttcaataaa cgattttggt 720
acacttgcag tcagggtagt taatgactac aacccagcca gggtggagac aaggattaga 780
gtatacatga agcccaaaca tgtgagagtc tggtgcccgc gacctccaag agcggtaagc 840
tacagaggac ctggagtcga cctcctatca acatcagtaa cacctttatc caaacatgac 900
ctagcgacat ac 912

<210> 32
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 32
```
ggagatacag tgagtgatat gatcgaaaat tccatcaacc gaattaccag tgcaatttcc      60
actacccaga cacaccagac agcagctgac actagagtta gtacacacag gttaggcacg     120
ggggaggtgc cacctttaca agcagcagag acaggtgcca cctccaacgc aaccgacgag     180
aacatgattg aaacacgctg tgtcgtcaac aggcacgggg tgagcgagac cagcgtggaa     240
tacttcttct ctcgctctgg tttggcagga atagtcatcg tggaggatgc aactgccact     300
aataagggtt atgccacatg ggagattgat gtcatggggt tcgcgcaact gcgtcgcaag     360
ctggagatct tcacatacat gcgcttcgat gcagagttca cttttgtggc aacagaacgc     420
aatgggagca ccagcccggt catgatgcag tacatgttcg tgccccctgg cgcccctgtt     480
ccaacaggga gagatacctt ccaatggcaa tctgctacta acccttcagt gctagtaaaa     540
atgacggatc caccggccca agttgccatc cccttttatgt ctccagctag tgcataccaa    600
tggttctatg atggatatcc tacctttgga gaaagaccag ttacaaccaa catgaattat     660
ggacagtgtc ccaacaacaa aatgggaact ttttgtatac gcactgtctc cggtgaagcg     720
tcagggaaaa acatcactat acgtattttt atgaggttga agcatgtaag agcgtgggtg     780
cctcgcccaa ttagaagcca gctatatctg cttaaaaatt accccaactt tgataacact     840
aagatcctca acgcctccca caacagagct tctatcacat caaacaca                  888
```

<210> 33
<211> 927
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 33
```
gggttggaag atctaataca acaagttgcg tctaacgcat tacaattgtc ccagccaaca      60
agaccggcac tcccaccagc cgagcagagt gtccccaaca ctaaccaaac aactccagaa     120
cactccaagg aagtcccagc gttaacggca gttgaaactg gcgccacgaa tcctctagag     180
cctggcgaca cagttcagac tagacatgtg atacaaacta gaagtagaag tgaaagtaca     240
gtggagtctt tctttgcgcg aggtgcatgt gtaaccatta tgggagtgga caactataat     300
gagacattga aaggagacca gaagtctact ctatttacaa cctggaacat cacctacact     360
gacacagtcc agctacggag aaaactggaa atgttcactt actccaggtt tgacatcgag     420
tttacttttg tggtgactga acgctactac tcatcaaaca gtgggcatgc tctgaaccaa     480
gtgtaccaaa ttatgtatgt accacctgga gcaccagtgc caaagaaatg ggatgattac     540
acctggcaaa cctcttcaaa cccgtccata ttctacactt atgggtcagc accacccagg     600
atatccatac cctttgtggg tatagcaaac gcttactccc acttctatga tgggtatgcg     660
acagtgccct tgaaaactga caccacagac tcaggagcag cctactatgg agcagtatcc     720
ataaacgact tcggactgct tgcagttcgc gtcgtcaatg aacataatcc agtcagagta     780
tcatccaaaa ttagagtgta tatgaaacca aaacatgtca gggtatggtg tcccagacct     840
ccaagggctg tagagtatta tggaccagga gtggactaca aggcaaacac tttaacaccg     900
ttgccaataa agaatttgac tacttat                                         927
```

<210> 34
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 34
ggtgacaaag tggcagacat gattgagacc gcagtggaga agaccgtgtc ctcactaact 60
tcccctattc aaaccccac agccgccaac acaaacgtga gtaatcatcg aattgagctg 120
ggggaagtcc cggctttgca agctgctgaa accggcgcga cgtctcttgt gtctgatgaa 180
tacttgatag agactcgttg tgtagtgaat agccatagta cagaggaaac tacagtgggg 240
cacttctttt caagagcggg gttggtggga gtgattgacc tcccattaca gggaacagtc 300
aacacaggag gattcgcctc gtgggatatt gatgtaatgg gatatgttca gatgagaagg 360
aaacttgagc tgttcacata tgcccgcttc gatgcggagt ttaccttcat agcttccacc 420
ccagatggcg aggtgaagcc agtgttctta cagtacatgt tcgtcccccc tggtgcacca 480
aaaccaacag ggcgcaacac ctacgaatgg caaactgcaa caaacccttc tgtgttggtc 540
aagagcacag atcctccagc acaagtctct gtaccgttca tgtcaccagc cagcgcatat 600
cagtggttct atgacgggta cccaaccttt ggaaagcacc tgcctgctga tgactttcag 660
tacggtatga ccccaaataa catgatggga tcgttctgtg ccaggatagt gggggaagga 720
gcgcctagtg tacacttggt tatccgtatc tacatgcgca tgaaacacgt gcgggtgtgg 780
attccacgac ctatgcgcag ccagccatac gttgcgaaga attaccctaa ctacaagggt 840
tctgagatca agtgcgcatc atctagtcgt aagtcaatca ccacatta 888

<210> 35
<211> 912
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 35
gggccaatag aggagatcat ctcgaccgtc gccagcaatg cacttgccct cagtcagcct 60
aaaccggtgg ataattctgt acaaaacacc caacagagcg cgcccgtgca cagccaagag 120
gttccagcat taacagcagt agagactgga gcaacaagtg atgtggtgcc agctgatcta 180
gtgcaaacca ggcatgtagt gaatgtcaag tccagatctg agtccactat cgagtcgttc 240
tttgcaagag ctgcctgcgt gactattatg caggttgata actttaatgc caccaccacg 300
gaggacaaga ggaagttatt tgccaaatgg gccatcacat acacagacac agtacaattg 360
aggaggaaat tggaattttt cacgtactcc aggttcgatc ttgagatgac tttcgtgcta 420
actgaaagat actattctca gagctcggga cacgctagat cgcaggtgta tcaaatcatg 480
tacgtccctc caggagcacc aacaccaaat gcatgggatg attacacgtg gcagacgtct 540
tctaacccat caattttctt caccactggt aacgcacccc cacgggtttc aatcccattt 600
gtgggcattg caaatgctta ctcacacttt tatgatggct tcagcagggt acctttggaa 660
ggagagacca ctgactcagg tgacgcttat tatggcctca cttctatcaa tgactttgga 720
acacttgcag taagagtggt caatgactac aacccagcga gagtggagac aaggatcaga 780
gtctacatga aacctaagca tgtgagagtg tggtgtccac gaccccctag ggctgtgagc 840
tacagaggac ccggtgtgga cctactgtcc acctcagtga cgcccctatc taagcatgaa 900
ttgacaacgt ac 912

<210> 36
<211> 918
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 36
ggcattgaag acttgatcca acaggttgca tcgaatgcgc tgcaaatctc acagccgacg 60
cgtccggcac tgccctctac agaaagtctt cccaacacac aacaatcggc accttcgcat 120
tctcaagagg tcccggcgct gacagcagtt gagacaggcg cgacaaatcc attggagccg 180
tctgacacgg tacaaacaag gcatgttatc cagactagat ccaggtcaga gtccacaata 240
gagtccttct tcgcgcgtgg tgcatgtgtg acaatcatga cagtggaaaa ttttaacgcg 300
actgaggcgg cagacaagaa aaagttgttc gccacttgga atattacata cacagacaca 360
gtgcagctca gaaggaagtt ggagatgttc acttactctc gatttgacat tgaatttacc 420
tttgtcacca cagaaaggta ctacgccagt aactcaggcc atgcgcgtaa tcaggtttac 480
caactcatgt atgtaccccc aggagcccct gtgccacaac aatgggatga ttacacgtgg 540
caaacttcct ccaacccatc ggtgttttac acatacggtg acgctccagc gcgcatttcc 600
ataccatttg tagggatagc taatgcctat tcccactttt atgacggcta tgcagtggtg 660
ccattgaaag attccaccca ggatgctggt gctgcctatt atggtgcaac ctcaattaat 720
gattttggaa tgttggcggt gagagtagtc aacgaattca acccagccag aatcacatct 780
aaattgagag tgtacatgaa accaaagcat gttagggtgt ggtgtcctag accaccaagg 840
gtggtgccgt acttcggacc cggtgttgat tataaggata gtttgacacc gctttctaca 900
aaagcactca acacttat 918

<210> 37
<211> 927
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 37
ggcttggaag acctcatcca acaagtggcc acgaatgcat tgagtctgtc gcagcccaca 60
agacccgcac ttccaccagc agaacaaagt gtgccaaaca ccagtcagac caccccagaa 120
cattcaaagg aagtacccgc actcactgca gtggagaccg gtgcaaccaa cccattggaa 180
ccaggtgaca cagtgcaaac tagacatgtt gttcaaacaa gatcaaggag cgaaagtacg 240
gtggaatctt tctttgcaag aggggcgtgt gtcacgatta tgggagttga caattacaat 300
gaaagcttga ccagtagtca aaaatccacc ctattcgcca cttggaatat tacatacact 360
gatacagtac agttgaggag aaaattggaa atgttcacct actccagatt tgacattgaa 420
tttaccttcg tagtaactga acgttactac tcgtcaaaca gtggccatgc cttgaatcag 480
gtgtatcaaa tcatgtatgt gccaccaggc gctccaattc ctaagaagtg ggatgattat 540
acctggcaaa catcatcaaa cccctcaata ttctacacct atggaacagc accacccaga 600
atttcgatcc cttttgtggg cattacaaac gcgtactcac atttttatga cggatatgcg 660
actgtaccac tcaagacaga cactacggat ccgggggcgg ccttctatgg agcagtttcc 720
atcaatgact ttggtttgtt ggcggtgcga gttgtcaacg agcacaaccc ggtaagagtg 780
tcttcaaaga taagagtgta catgaagcct aaacatgtca gagtgtggtg cccacgacca 840
ccacgtgccg tggagtacta cggaccaggg gtagattaca aggcaaacac attgacacct 900
ctccctacca agaacttaac tacttat 927

<210> 38
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 38
ggtattgatg atatcataga taatgttgta accaatgctt tgaaggtgtc catgccacaa 60
gttcaagata cgcaatctag tggaccagtt aactcaaaag aagtacctgc attaacagct 120
gttgaaacag gggctactag tcaagttgac ccatcagacc taatagaaac tagacatgtt 180
attaataacc gcctcagatc tgagtgcaca atagaatcat tctttgggag gtcagcatgt 240
gtggccataa ttgggttatc taaccaaaaa cccaccagtg acaatgcagc caagctcttt 300
gctacatgga agattagtta tcttgatatg tatcaattga gaagaaaatt ggaattcttc 360
acatactcca gatttgatct tgagttaacc tttgtaattt cagaaagatt cttcacctca 420
acttcagctg ctgcaagaga ttatgtatac cagatcatgt acattccccc aggagcccct 480
atccctcagg tatgggatga ttacacatgg caatcatcca caaacccctc aatattctac 540
accacaggaa atgcatgccc tagagtgtcc atccctttttg ttgggatcgg tgcagcatac 600
tctcacttct atgatggatt ctctttagta cctttcaata ccatcgatgc tggtgcttca 660
aacaggtacg ggtacaccac cataaatgat tttgggacta tggcaatcag gatagttaat 720
gaatacgacc cagtcacaat tgatgcaaaa gtcagggttt acatgaaacc aaagcatatt 780
aaggtgtggt gccccagacc tccacgggca gtagcataca atgggccaac agtgaatttt 840
aatgaaaacc cccatgtaat gacagcagtt gctgatatta gaacttat 888

<210> 39
<211> 909
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 39
ggtatcgaag atcttatcac cgaagttgca agcaacgctc tgaagttgtc acaaccaaaa 60
cccagcacac aacagagttt accaaacact agtagctcag aaccaactca ctctcaggaa 120
gcgccggcat tgaccgcagt agaaacagga gcaactagta gcgtagtacc agctgatctg 180
gtccagacgc ggcatgtgat acaaacacgt agccgaagtg agtctacagt tgagtcattc 240
tttgctcggg gggcgtgtgt aacaatcatg tcagtggaaa attacaatga aaccgctatc 300
gcagagtcca aattatttac caagtggaac attacctaca cagacacagt ccagttgaga 360
agaaaactag agatgttcac atactccaga tttgatattg agttcacatt tgtggtgact 420
gagcgttacc actccgcaaa ctcaggtcat gcactaaatc aagtttacca gatcatgtat 480
gttcctccag gtgcaccagt gccacaaaga tgggacgact acacatggca aacgtcatcc 540
aacccctcag tcttttatac ctatggtaca gcaccagcca gaatatcgat tccatatgta 600
ggcatagcca atgcctactc gcatttttat gatggcttcg ccaaagtgcc cattgaaggc 660
gagacgtcag atccaggtga tgcatactat ggtgcaacgt ccatcaatga tttcggcatc 720
ttagccatac gtgtggtcaa cgaacacaat ccagtgcaag tttcttccaa gattagagtg 780
tacatgaaac ctaaacatgt gcgcgtttgg tgtcccagac cacctagagc tgttccatac 840
tttggccccg gggttgatta taaaggtgac gccctcacac cactatcacg caaggattta 900
accacctat 909

<210> 40
<211> 888
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 40
gggattgagg atacaatcga aaaagtggtt ggtgatgctc taagggtctc aatgccacaa 60
gttgccaaca cccagccatc aggacccgta aattctaagg aagttccagc actgacagca 120
gtggaaacag gtgcaaccag tcaagtcacc cctgaagatt tgatcgaaac caggcatgtt 180
attaacaata gactaagatc tgagtgcact gtggaggcct tctttggaag gtctgcatgt 240
gttgccatcc ttggtgtggt aaacaaaaag ccagacacca caaatgccaa agacctcttt 300
acaacatgga ggatcactta cctgcaaact tatcaactga ggaggaaact cgaactcttc 360
acgtattcta gatttgattt ggaattaacg tttgtcatta cagaaagata cttttcaggg 420
acagcagcca caaccagaga ttatgtttac caaataatgt atgtaccacc aggagccccc 480
ataccaaata cctgggacga ctacacctgg cagtcatcta ccaacccctc tgtcttctac 540
accacaggca atgccagccc acgcatgtct ataccctttg ttggtattgg tgccgcctat 600
gctcactttt atgacgggtt cagtgtggta ccattcaatc aaatagatgc aggagcatcc 660
aacaaatatg gctactcatc aatcaaagac tttggtacat tggcagttag aattgttaat 720
gagtttgatc cagtgacaat agaggctaaa gtcagagtgt acatgaaacc caaacatgtc 780
agggtgtggt gtccaagacc acctcgtgca gtaccatatc aaaactcatc agttgatttc 840
gcccaaaacg cagtagcaat gaaccaagta gccacaatta ggacgtat 888

<210> 41
<211> 915
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 41
ggtatcgaag ataccattga cactgtcatt aacaatgccc tacaactatc tcaaccacag 60
ccaaataagc agttgacagc tcagtctacc ccctccacaa gtggagtaaa ctcccaggag 120
gttccagctc tgaccgctgt ggaaaccggt gcctcgggac aagcagtgcc cagtgatgtg 180
attgagacca gacacgtggt taattataag acccgatctg aatctactct tgagtctttc 240
tttggaaggt cagcttgtgt caccataatt gaggtcgaga acttcaatgc cactagtgaa 300
gcagacaaga ggaaacagtt caccacttgg ccaatcacat acaccaatac cgtgcaattg 360
cgcaggaaac tagaattctt cacttactcc aggtttgacc tagagatgac ctttgtagtg 420
acagaaagat attatgccag caacacaggt cacgccagaa accaagtgta tcaaataatg 480
tacattcctc ctggtgcacc acaacccaca gcatgggatg attacacgtg gcaaagctct 540
tcgaatccgt cagtctttta cacttatggg agtgctccac ccaggatgtc tataccgtat 600
gtcggtatcg caaatgcata ctctcttttt tatgatgggt ttgcacgagt accactgaag 660
gacgaaacag cggactcagg tgatactttt tacgggctag tcaccatcaa tgattttgga 720
accttagcaa taagagtagt gaatgaattt aacccagcta ggattacatc aaaaattaga 780
gtgtatatga aaccaaagca tgtaagatgc tggtgcccta gaccaccacg tgcagtgcca 840

taccgtggtg aaggagtaga ttttaattca agttcaatca caccactaac agcagtcgca 900
aacatcaaca cattc 915

<210> 42
<211> 852
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 42
agcccagtgg aggaatccat tgagagaagc attggcagag ttgctgacac cattggtagt 60
ggaccatcca attcggaggc aataccggca ctcacagcag tagaaacagg acacacatca 120
caggttacac ctagtgacac gatgcaaaca agacatgtgc acaactacca ttcaaggtcc 180
gaatccagcg tagagaactt cctggcacgc tcggcttgtg tgttttatac aacatacacc 240
aacggtaaaa aaaaaaatgc cgccaaagag aagaagtttg caacgtggaa agtgagtgtt 300
agacaagccg cccaactaag aagaaagcta gagttattca catacttacg ctgtgacatc 360
gaattaacat tcgtcatcac cagtgcacaa gatccatcga ccgctaccaa cttggatgtg 420
ccagtgttga cccatcaaat aatgtacgtc ccacctggtg gtccagtccc tgaaaccgtg 480
gacgattaca actggcaaac atctacaaat cccagccttt tttggactga agggaatgca 540
cctccacgca tgtcaattcc attcatgagc ataggcaatg cctatagtat gttctatgat 600
ggttggtccg agtttaggca tgacggtgtg tacggcctga ataccottaa caatatgggc 660
acaatatatg ctaggcacgt caacgctgac aacccaggta gcatcaccag cacagtgaga 720
atatacttca aacccaaaca tgtcaaggca tggattcctc gcccgcctcg tttggcacag 780
tatcttaaag ccaataatgt gaattttgag atcaccgatg tgacagaaaa gagagatagt 840
ctcacgacca cg 852

<210> 43
<211> 846
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 43
agcccagtgg agggcgccat agagagagcc attgcacggg tcgctgacac tatgccaagt 60
ggcccaacca attcagaagc agtgcctgcc ctgacagcag tggaaacggg ccacacctcc 120
caagtcgtcc ccagtgataa catgcaaacc aggcacgtga agaagtacca ttcacgctcc 180
gaaaccagcg tcgagaactt tctgtgtagg tctgcatgtg tatattttac cacatataag 240
aaccagacag ggcgaaaaaa tagatttgct tcttgggtaa tcaccacaag acaagtggcc 300
cagctcagga gaaaactaga aatgtttacg tacttgcgtt tcgacattga actcaccttt 360
gtcattacaa gtgcgcaaga ccaatccact atttcccaag acgcccctgt gcagacacat 420
cagataatgt acgtgccacc gggaggccca gtgccaacca aagttgacga gtatgtgtgg 480
caaacatcca ccaaccccag cgtcttttgg accgagggta acgctccacc acgtatgtca 540
gttcccttta tgagtatcgg taatgcttat agcacatttt atgacgggtg gtctgatttt 600
tcaaacaaag gaatatatgg gttgaacacc ttgaacaaca tgggaacatt gtacatccgc 660
cacgttaacg ggcccaaccc agtaccaatt accagcacag tgaggatata ctttaagccc 720
aagcatgtta aggcctgggt gcctaggcct ccaaggcttt gccagtacaa aacgtttagg 780

caagtcaact ttacagtgac tggagtgacc gagagtaggg caaatataac caccatgaat 840
actaca 846

<210> 44
<211> 852
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 44
ggtgatgtgc agaatgctgt cgaaggggct atggtcaggg tggcagatac agtgcaaact 60
tcagccacaa actcagagag ggtgcctaac ttgacagcag tagaaactgg tcacacttcg 120
caggtagtac ctggtgatac catgcagact agacatgtga tcaacaatca cgtgaggtca 180
gaatctacaa ttgagaactt ccttgccaga tcagcgtgtg ttttcttcct agagtacaag 240
acagggacca aagaggattc caatagcttc aacaattggg tgattacaac caggcgagtg 300
gctcaactac gtagaaaact ggaaatgttt acttacctac ggtttgacat ggaaatcacc 360
gtggtcatta caagctcgca agatcagtct acatcacaaa accagaatgc accagtgcta 420
acacaccaga taatgtatgt accaccaggg ggacccatac ccataagcgt ggatgattac 480
agctggcaaa catccaccaa ccccagtatc ttttggaccg aagggaacgc tccggcacgc 540
atgtcaattc catttattag cataggcaat gcgtatagta atttctacga tgggtggtct 600
cacttctccc agactggcgt gtatggcttc actactctga acaacatggg tcaattgttc 660
ttccggcacg taaacaagcc caacccagcc gctattacaa gtgtggcgcg catttacttc 720
aaaccgaaac atgtacgcgc ttgggtgcct agaccaccgc gcttgtgtcc atacatcaat 780
agcacgaatg tcaactttga acccaagcca gtgactgaag tacgtaccaa cataataaca 840
acgggtgcct tc 852

<210> 45
<211> 882
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 45
ggagatgagg tgaagcatga acccacagtg gccaacacaa cagcaagtgg accatcaaat 60
tcacaacaag taccggcact cacagcagtg gagactgggc acacctcaca ggtggttcca 120
agcgatacca tacaaaccag acatgttcac aattaccata gtagaactga atccaccctg 180
gagaacttcc tcggaagatc agcatgcgtg cacattgact cgtataagac caagggagtg 240
accggcgaga gcacccggta cgcatcatgg gagatcacca ctcgcgagat ggtgcagctg 300
cggaggaagt gtgaactctt cacctacatg cgatatgatc tagaaatcac gtttgtgatt 360
acaagtcgcc aggagcaagg ggccaaactg tcgcagaaca tgccagtatt aacacatcag 420
atcatgtatg tcccaccggg cgggcctata ccaaccagca acgagagtta cgcttggcaa 480
acgtcaacga acccaagcgt gttttggaca gaaggaagct cgccaccacg aatgtcaata 540
ccgtttgtta gcataggaaa cgcatacagc aatttctatg atgggtggtc gcacttctca 600
caaaacggtg cgtatggtta cacggcacta aacaagatgg gtaggatatt cgtgcgccat 660
gtaaacaaag agacaccact gcaagtcata agcacaatac ggatgtatat gaagcccaaa 720
cacgtgcggg cttgggtgcc aagaccacca cgcctgtgtc catacctgcg ggcgggtgat 780

ataaactttg aagtgactga tgttacagaa aaacgaaata acatcaatta tgtcccaacc 840
ccatcccaca gcagcagtgt gcacatgcgc ttgaacaacc at 882

<210> 46
<211> 879
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 46
ggggacgtcg aagaggcaat tgatagggca gttgcgaggg tggctgacac aatgccaacc 60
ggtccacgaa acactgagag cgtgcctgcc ctgacagcag tagagacagg ccacacctca 120
caggtcgttc ctggtgacac aatgcagacg aggcatgtta agaactatca ctccaggaca 180
gagtcatcaa ttgaaaactt cctgtgcagg gctgcgtgcg tgtatataac aacatacaaa 240
tcagctggtg gaacacccac agagcgatat gcaagttgga ggataaacac caggcaaatg 300
gtgcagctca ggaggaaatt tgagctcttc acatacttgc gctttgacat ggaaatcaca 360
tttgtgatca caagcacaca agatcctggg acacaattgg cacaagatat gcctgtacta 420
actcatcagc tcatgtatat cccacctggg ggccctgttc ctaacagtgc cacagatttt 480
gcatggcaat catcaactaa tccaagtata ttttggacgg aaggctgtgc tccagcacga 540
atgtcggtgc cgttcatcag cattggcaat gcctacacca atttttacga tgggtggtcg 600
catttcaccc aagaaggggt ttatgggttt aactcactga acaacatggg ccacatatat 660
gtgaggcacg tcaatgagca aagcctgggt gtctcgacca gcaccgttcg cgtgtatttt 720
aaacccaaac atgtgcgtgc ttgggtacca agaccaccca gactgtgccc atacactaag 780
agttcaaatg tgaatttcaa accgaccgct gtcactgatg agcgaaagga tatcaacgat 840
gtaggcaccc ttcgaccaac agtgtacact aaccttgtg 879

<210> 47
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 47
ggagacgtgc aagatgcagt gacaggtgct atagtacgtg tcgctgacac tctcccaaca 60
ggtccctcaa ataatgaagc tatacccaat ttaacagcag tggagactgg ccatacctcg 120
caagtgacac caggcgacac aatgcaaaca cgccatgtgg tgaacatgca cacccgctct 180
gagtcgtcca tcgagaattt cctggcacgt tcagcatgcg tgtactacct tgattaccaa 240
acgggagaag ggcccggcga tcagtatttt ggccagtgga ccattaccac gaggagggtt 300
gcgcaattgc gtcgaaagct ggagatgttc acttatctaa gatttgacat ggaaatcaca 360
atcgtgatta ctagttcaca ggatcaatct accatctcga acccagatac accagttttg 420
acgcaccaaa ttatgtatgt accaccagga ggaccaatcc cagcaaaagt cgatgattac 480
agttggcaaa catccacgaa tcccagcgta ttctggactg aagggaatgc gcctgcccgr 540
atatccatcc cattcattag cgttggaaat gcatacagta gcttttatga cgggtggtcg 600
aacttctcac aaaacgggcg gtatggctac aataccctca acaacatggg acaattgttc 660
tttaggcacg ttaacaaacc cagccctaat actgtcacaa gcgtcgcccg catatacttc 720
aagcctaagc acgtgagagc ttggatcccg cgaccaccgc ggttgtgtcc atacataaat 780

gcgggagacg tgaacttcac tccgacacca gtgactgaaa agcgaaagga cctaataacc 840
acg 843

<210> 48
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 48
ggagatgtgc aggacgcagt ggctggggcc atagtgcgtg tggctaatac tctcccatca 60
ggcccctcaa acaatgaggc tatacccaac ttaacagccg tagaaactgg acacacctcg 120
caggtgacac cgggtgatac aatgcagacg cgccacgtag tgaacatgca cactcgttct 180
gagtcgtcaa tcgagaactt cctggcgcgg tcagcatgtg tatactacct cgattaccga 240
acaggaacgg ggcctggcaa tcaatacttt agccagtgga ctattaccac aagacgagtt 300
gcgcagctgc gtcgaaaatt ggagatgttc acctatctaa ggttcgacat ggagatcacg 360
attgtaataa cgagttcaca agatcagcct accgtccgaa acccagacac accggtcttg 420
acacaccaaa tcatgtatgt gccaccagga gggccaatcc cagcaaaggt cgacgattac 480
tgttggcaaa catccacaaa ccccagtgtc ttctggactg aagggaacgc accagcccgg 540
atatccatcc cgttcatcag tgtcgggaat gcatatagta gtttctacga tggatggtca 600
aatttctcgc aaaatgggcg gtatggctac aacaccctga acaacatggg gcaattgttt 660
ttcaggcatg tcaataaacc cagtcccaac actgtcacaa gtgttgcccg catatacttc 720
aagcccaaac acgtgaaggc atgggtcccg cgaccaccgc gattgtgccc ttacattaat 780
gctggagatg taaatttcac ccccacatcg gtcactgaga agcgagcgag cctgataacc 840
aca 843

<210> 49
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 49
ggggacgtgc aagatgccgt gactggagcc atagtgcgtg tcgccgacac actgcacacg 60
ggaccctcga acaacgaagc aatacccaat ttgacggccg tggaaacagg gcatacatcg 120
caagtgacac caggcgatac aatgcagacg cgtcacgtgg tcaacatgca cacccgttca 180
gagtcatcaa ttgagaactt cctagctcga tctgcgtgtg tgtattacct cgactatcaa 240
acagggtcag gacctggcac ccaatacttc ggccagtgga ccatctccac aaggagagtt 300
gcgcaactgc gccggaagtt ggaaatgttc acctacctaa gatttgacat ggaaataaca 360
atcgtgatca ccagttcgca agatcactcc accatctcaa atccagatac accaatcatg 420
acgcaccaaa ttatgtacgt accaccaggg ggtccaatcc cggcgaaggt cgacgactat 480
agctggcaaa catctacaaa ccctagtgta ttttggacag aagggaacgc acccgcccgc 540
atatccattc cattcattag tgtcggaaat gcctatagca gcttctacga cgggtggtca 600
aatttctcgc aaaacggccg atatggatac aacactttga acaacatggg acaactattc 660
ttcagacacg tgaataagcc cagccccaac accttcacaa gtgttgcccg tgtatacttc 720
aagccaaaac acgtgaaggc gtggattcca cgaccaccgc gattatgtcc atacataaat 780

gcgggagacg tgaatttcaa accaacaccc gtgaccgaaa agagggcgag cttaatcacc 840
aca 843

<210> 50
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 50
ggagactcag agcacgcagt ggaaagcgcc gtatctaggg tggcagatac aattatgagt 60
ggcccgtcaa actcccaaca ggtccccgct cttactgcag ttgaaactgg acacacatcg 120
caagttgttc caagtgatac catccaaacc agacatgtgc agaatttcca ctctaggtcc 180
gagtcgacca ttgaaaattt cctgagtagg tcagcatgtg tgcatatcgc caattacaac 240
gcgaagggcg ataagacgga tgtggacagg tttgacaggt gggagatcaa cattcgtgaa 300
atggtgcaac tacgtaaaaa gtgtgagatg ttcacatatc tacgctatga tattgaagtt 360
acatttgtta taaccagcaa acaggatcag ggccccaaac taaaccagga tatgcctgtt 420
cttacccacc aaattatgta cgtacccccca ggaggttcag tacctagcac cgttgagagc 480
tatgcgtggc aaacatcaac aaaccctagc gtgttttgga ccgaggggaa cgctccagct 540
agaatgtcca taccctttat cagcataggg aacgcttata gtagcttcta tgatggatgg 600
tcacacttta ctcaaaaagg ggtctacgga tacaacacat taaacaagat ggggcagcta 660
tttgtcagac atgtgaacaa acagaccccc acgccagtta ctagtaccat aagggtttac 720
ttcaaaccaa agcacattag agcttgggtc cctaggcccc cgcggttatg cccctatgtg 780
aacaagacaa atgtaaactt catcaccaca caggtaacag aacctacaaa tgacctcaat 840
gacgtgccca agtctgagca taacatgcac acatat 876

<210> 51
<211> 867
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 51
aacgacgttc agaacgcggt ggaacggtca attgttcgtg tagcggacac attacccagt 60
gggccaagca actcagaaag cataccagca ctcacagcag ccgagactgg acatacctcg 120
caggtcgtcc ccagcgacac catccagacg cgacatgtga ggaattttca cgttcggtct 180
gagtcatcgg tagagaattt tcttagcagg tcagcttgcg tgtacatcgt ggagtacaaa 240
acccgggaca cgactcccga caagatgtat gatagctgga ttatcaatac caaacaagtg 300
gcgcagttga gaaggaagct ggagttcttt acctatgtca gattcgacgt ggaagttacc 360
tttgtcataa ccagcgtgca agatgactcc acaaaacgga acaccgacac cccagtgcta 420
actcatcaaa ttatgtatgt gccgccagga gggcccatac cacaagcggt ggacgattat 480
aactggcaaa cttccaccaa ccccagcgta ttttggactg aggggaacgc gccaccaagg 540
atgtctattc cgttcatgag tgttggcaat gcatacagta acttctacga cgggtggtcc 600
cacttttctc aaactggggt ttacgggttt aacaccctaa acaacatggg taagttatat 660
ttcaggcatg taaacgacag gactattagc ccaatcaaaa gtaaggtcag aatatatttc 720
aaacccaaac acgtgaaggc atgggtaccc agaccgccga gattgtgtga atacacccac 780

aaggataacg tggactatga accaaagggg gtcacaacat cacgcacttc aatcaccatc 840
accaactcca cacacatgga gacgcac 867

<210> 52
<211> 867
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 52
aatgacgttc aaaatgcagt cgagcaatca attgttcgtg tggctgacac gttacccagt 60
ggacccagta attcagagag cataccggca ctgacggccg ccgagactgg ccatacttct 120
caagttgtgc ccagtgatac tatacagaca cgccacgtaa aaaactttca tgtgaggtcg 180
gagtcgtcag tagagaactt tctcagtagg tccgcttgcg tgtatatagt gggatacaag 240
accacagatg cgacccctga caaaatgtat gacagctggg ttatcaacac aaggcaggtg 300
gcgcagctaa ggagaaaatt agagttcttc acctatgtta ggtttgatgt tgaggtcacc 360
tttgtgataa caagcgtgca agacgattca actagacgga acacagacac ccccgttcta 420
acccaccaaa tcatgtacgt acccccaggt gggcccatcc cgcaggcagt ggacgactac 480
aattggcaaa cttccacaaa tcccagtgta ttttggacag aagggaatgc cccaccaaga 540
atgtccatac cattcatgag cgtaggtaac gcatacagca atttctatga tgggtggtct 600
cacttctctc aaactggggt gtacggtttc aacaccctga acaacatggg caagctatac 660
ttcaggcatg tgaacggcaa gacaataagc cctatcgcaa gcaaggttag gatttacttc 720
aaaccaaagc atgtgaaggc atgggtgccc agaccaccgc gattgtgtga atacacccac 780
aaggacaatg tggattacga accaaaggga gtcacaacat cccgtacatc tatcacaatt 840
agcaattcca ctcatatgga aacatat 867

<210> 53
<211> 867
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 53
aacgacgttc agaacgcggt ggaacggtca attgttcgtg tagcggacac attacccagt 60
gggccaagca actcagaaag cataccagca ctcacagcag ctgagactgg acatacctcg 120
caggtcgtcc ccagcgacac catccagacg cgacatgtga agaattttca cgttcggtct 180
gagtcatcgg tagagaattt tcttagcagg tcagcttgcg tgtacatcgt ggagtacaaa 240
acccatgaca cgactcccga cgagatgtat gatagctgga ttatcaatac cagacaagtg 300
gcgcagttga gaaggaagct ggagttcttt acctatgtca gattcgacgt ggaagttacc 360
tttgtcataa ccagcgtgca agatgactcc acaagacaga acaccgacac cccagtgcta 420
actcatcaaa ttatgtatgt gccgccagga gggcccatac cacaagcggt ggacgattat 480
aactggcaaa cttccaccaa ccccagcgta ttttggactg aggggaacgc gccaccaagg 540
atgtctattc cgttcctgag tgttggcaat gcatacagca acttctacga cgggtggtcc 600
cacttttctc aaactggggt ttacgggttt aacaccctaa acaacatggg taagttatat 660
ttcaggcatg taaacgacag gactattagc ccaatcacaa gcaaggtcag aatatatttc 720
aaacccaaac acgtgaaggc atgggtaccc agaccgccga gattgtgtga gtacacccac 780

aaggataacg tggactatga accaaagggg gtcacaacat cacgcacttc aatcaccatc 840
accaactcca cacacatgga gacgcac 867

<210> 54
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 54
ggcgacaccg aaacggctat tgacaatgca atcgccaggg tagcagatac ggtggcgagc 60
ggtcctagta attcgaccag tatcccagca ctcacagcag ttgagacagg tcacacgtca 120
caagtcgagc ccagcgatac agtgcaaact agacatgtca aaaactacca ctcgcgttct 180
gagtcaaccg tggaaaactt tctaagtcgc tccgcttgtg tgtacatcga agagtactac 240
accaaggacc aagacaatgt taataggtac atgtcgtgga caataaatgc cagaagaatg 300
gtgcaattga ggagaaagtt tgagctgttt acatacatga gatttgatat ggaaatcacg 360
tttgtaatca caagtagaca actacctggg actagcatag cacaagatat gccgccactc 420
acccaccaga tcatgtacat accaccaggt ggcccggtac caaacagcgt aacagatttt 480
gcgtggcaga catcaacaaa ccccagtatt ttctggacag aaggaaacgc gccacctcgc 540
atgtctattc cattcatcag tattggcaat gcatatagca acttctatga cgggtggtca 600
cacttttccc aaaacggtgt gtacggatac aacgccctga acaacatggg caagctgtac 660
gcacgtcatg ttaacaagga cacaccatac cagatgtcaa gcacaatccg agtgtatttc 720
aaacccaagc acatccgagt atgggtccca cggccgcctc gactgagccc gtacatcaaa 780
tcaagtaatg taaattttaa ccccacgaac ctgacggacg agcggtcatc catcacatat 840
gtgcccgaca ctatacgtcc agatgtgcgc accaac 876

<210> 55
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 55
ggtgatgtcc agaatgcagt tgagggggca atggttagag ttgcagatac cgtgagcact 60
agcgccacca actccgaaca agtgccgaac ctgaccgcgg tggagaccgg tcacacatcg 120
caggtagtgc ccggcgacac tatgcagacc aggcacgtag tgaacaagca tgtgcgatct 180
gaatctacaa ttgaaaattt cctcgcacgt tcagcctgtg tgtactttct tgagtacaag 240
actggtacca agactgactc caacgccttc agcaattggg tcatcacaac gcgcaaggtt 300
gcgcagctga ggcgcaagtt ggagatgttt acatacttaa ggtttgatat ggagattact 360
gtggtcatta ctagctccca agaccagtcc acatcacaaa atcaaaatgc gcccgtcctg 420
actcaccaga ttatgtatgt accacctggt ggcccagtgc ccactagcgt tgatgattat 480
tgctggcaaa catccacaaa cccaagcata ttttggacgg aaggaaacgc acctgccaga 540
atgtccatcc cctttatcag cattggaaat gcttatagca acttttatga tgggtggtca 600
catttctcac agaacggagt ctatggtttt accaccttaa acaacatggg ccagctgttt 660
tttaggcatg ttaacaagcc taacccggcg acaataacca gtgtggcccg catttacttc 720
aagccaaaac atgtgagggc ctgggtgcct agaccgccac ggttgtgccc ttacatcaac 780

agtagcaacg tgaacttcga cccaaaacct gtggcagagg tcaggtctag catcatcacc 840
acc 843

<210> 56
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 56
ggtgatgtgg ttgaagccat tgagggcgca gttgctagag tagcagacac tatcagcagc 60
ggcccaacaa attctcaagc agtcccagca ctcacagcgg tggagactgg acacacctcg 120
caagttgtac caggtgatac catgcagacc agacacgtaa agaattacca ctcacgatca 180
gaatcgacca ttgaaaattt tctgagtagg gcggcttgtg tctacatggg tgagtattac 240
actacaaata cagatgagac caagagattt gctaattgga caatcagcgc aaggcgcatg 300
gtacaaatga ggaggaagct tgaaatgttc acgtacgtcc gtttcgacgt ggaggtgaca 360
ttcgtaatta ccagcaaaca ggaccaaggg aatcggttgg gacaagatat gccccccgctc 420
acacaccaga taatgtacat cccgccaggt ggtcgtatac ccaaatccac cacagattac 480
gcatggcaaa cgtcgacaaa ccccagcatc ttttggacgg agggtaacgc gccccccagg 540
atgtccattc ctttcatgag cattggaaac gcatatagca atttttatga cggttggtct 600
cacttctctc aaaatggcgt gtacggatat aacacactaa accacatggg tcaattatac 660
atgcgccatg taaatggacg atcacctctt ccaatgacca gcacggtgag ggtgtacttc 720
aaacccaaac atgtgaaaac atgggtgcca cgaccccccaa gattgtgcca atacaaaaac 780
gcctcgacag taaacttttc acccacaaac atcacagaca agagggatag catcacttac 840
attccagaca ccgtgaaacc cgacatgaca acatat 876

<210> 57
<211> 861
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 57
ggggatgaga gtgcaaaggc tacagtttcc aacacacagc ctagcggtcc aagtaattct 60
gtcagcgtgc caatgcttac tgctgctgag accgggcaca catctcaagc agtacccagt 120
gacactatac agaccaggtg cgtagtgaac caacacaagc ggtcggaatc atccgtggaa 180
aatttcctgt gtcgctccgc ttgcgtatac tacacaacct atgacactca cggggatgca 240
gccgacgcaa agtacgccag ttggacgata accaccccgaa aagctgcaca gctgcggaga 300
aaactagaga tgttcacata cttgaggttt gatttagaag tgacattcgt tataacaagt 360
gcacaagtaa catctaccaa taaacgtcag gacacgcctg ttctcacgca tcaagtcatg 420
tacgtgccac caggtggtgc agtacccgct agtgtggacg attatgcgtg gcagacgtcc 480
acaaacccaa gtatcttctg gacggaaggg aatgcaccag cacgcatgtc tataccctttt 540
atcagcgtgg gcaacgcata cagtagcttc tatgatgggt ggtccaactt tacacagaat 600
ggagtttacg ggttcaacac gctaaacaac atgggaaagc tatacgtacg acacgtcaat 660
ggagctagcc ccggccctgt gaagagtacc atacggtttt acatgaagcc caaacacgtg 720
aaggcttgga tacccagacc tcctcgcctc tgcgagtacg aaaaatcagg caatgtaaac 780

ttcaaaccca agggcgtgac agagagccgg acgtctatca aattagaaaa accaaacccct 840
gcgtccaaat taatgaacca c 861

<210> 58
<211> 894
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 58
aatgatccag agcaagctat aaatcgggcg ctagcgaggg tggcagacac agttcgtagt 60
gggccgtcta actctgaaca aattcccgca ctgacagccg tggagacagg gcatacatca 120
caagtcgtcc ccagtgacac aatgcaaacc cggcatgtga agaattacca ctccaggtca 180
gagtcaacaa tagagaactt tttgtgtaga tcggcttgcg tgcacatcgc aacatacaag 240
gctaaaggcg gagctggaga cgtcgaccgg tacgacagct gggacataaa cataaaagag 300
ctggtacagt tgcgacgcaa gtgcgagatg tttacgtacc taaggtttga tatggaggtc 360
acctttgtga ttaccagcat acaggagcag ggcaaagcac tgacccagga catgccggtg 420
ctaacgcacc aaataatgta cgttccaccg ggcggtgccg tgcctagtgg tgcagaaagc 480
tttgcgtggc agtcatcaac gaatcccagt gtgttctgga cagaaggcaa tgcaccagca 540
cgtatgtcta taccctttat aagtattggg aacgcttaca gtaatttcta tgatgggtgg 600
tcccacttta cccagaacgg tggttacggg tacaacacac taaacaaact gggtaagatc 660
tacgtcaggc atgtgaacaa acaaaccccc acggatgtca ccagcaccgt gcgaatttac 720
ttcaagccca aacacgtgcg agcttgggtg cctcgcccgc ctagactatg tccttataag 780
aacaaggcaa atgtaaactt tgaagttact agtgtaacca ctgccagaac gagtcttaat 840
gatgtcccca ctcccaacca cagtagtagc gtgcacctgc gcatgcacac gcac 894

<210> 59
<211> 882
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 59
ggtgatgacc aacacaagac caatacagtg acagacacag agcagagtgg cccgtcaaat 60
tccgaacgcg tcccagccct cacagcagtg gagactggcc acacttcgca ggtcgtaccc 120
agcgacacag tgcaaactcg ccacgtacgc aattaccact caaggacaga gtctacctta 180
gagaattttc ttggtaggtc agcatgtgtg cacatcgaca catacaaggc taagggtgaa 240
aaaggatctt ctgagaggta cgcgtcatgg gagataacta acagggagat ggtgcaattg 300
cgccgaaaat gtgagatgtt cacatatatg aggtatgacg tggaaataac atttgtgata 360
accagctacc aggagcaggg cacacgattg gcccaggaca tgcctgtact aacacaccaa 420
atcatgtacg tgcccccggg tgggcctgtg ccaacaagca cggagagcta tgcatggcag 480
acctcaacga accctagcgt cttttggact gagggcaacg caccaccgcg tatttccata 540
cccttcatca gcataggaaa tgcgtactgc aacttttatg atgggtggtc acatttctca 600
caagatgggt cctatggcta cacagcgctc aatagaatgg ggaaaatata tattagacat 660
gtaaataagg agacccccac acaggtcatt agtaccgtga ggatgtacat gaaaccaaaa 720
cacattcgcg catgggtgcc cagacccccc cggctgtgca aatacctaca ctcaggcaac 780

atgaacttca acgtggagga cattacagag gagcggaacg atataaacca tgtacccacc 840
cccagccaca gcagtagtgt gcgtgtgcgt cttggcacca ca 882

<210> 60
<211> 867
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 60
ggtgatgttg aggactcagt aaacagagca gtggttaggg tagcagacac catgccaagt 60
ggaccatcca attcgcaggc agtacctgcc ttgacagccg ctgagacagg tcacacgtct 120
caagtggtgc ctggtgataa catccaaaca cgtcatgtgc acaactacca ctccagaact 180
gaatccagta tcgaaaattt cttcgggcgt tccgcatgtg tagtggtcaa aacatataaa 240
atgggtcaaa aagttgtagc tacagacaga tatgatagtt ggatgatttc cattagggac 300
atggtacaac taagacggaa gtgtgaaatg ttcacgtaca tgagatttga tttagagatc 360
accttcgtgg tcacgagtta ccaacaatat agtacatcct tgacacagga catgccagtg 420
atcacgcatc agttcatgta tgtgccgcct gggggtccgg ttcctgagag tgtaaatagc 480
tacgcttggc aaacgtcaac caatcccagt atattctgga ctgagggtaa tgccccagca 540
aggatgtcca ttcccttcat cagtgttggg aacgcatata gctgcttcta cgatggctgg 600
tcacacttca cacagaaggg ggtttatggt tataacactc tcaacaacat gggcaaattg 660
tacatgcgac acgtgaacaa aaatagcccc acagagatca taagcactct tcgtgtgtat 720
ttcaagccaa agcacgtgaa agcgtgggta cccagaccac ccaggctatg tccatacaaa 780
tataaggcaa atgttgactt tgaagtgact ccaatcacag acaagcgaga ctccataacc 840
agcataccag tccccaagca cactcat 867

<210> 61
<211> 861
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 61
ggggataacc aggatcggac ggtcgccaac acacagccta gcggtccgtc caactccacg 60
gaaattccag ccttaacagc ggtggaaacg gggcacacct cacaagtgga tcccagtgac 120
actatccaga ccaggcacgt ggtaaacttc cactcacgtt ctgagtccac tatagaaaat 180
ttcatggggc gtgcagcatg tgtgttcatg gatcagtata aaatcaatgg agaagagacg 240
tccactgata ggttcgcagt gtggaccata aacataaggg agatggccca attaagaagg 300
aagtgtgaaa tgttcacgta catgcgtttt gatatcgaga tgacaatggt cattaccagc 360
tgtcaagacc agggaacgat actagatcag gacatgcctg ttttgacgca tcaaattatg 420
tacgtcccac caggggggcc aatcccagcc aaagtagata gttacgagtg gcagacatca 480
acaaacccca gcgtcttctg gacggaaggt aatgcaccac cgcgtatgtc tattccattc 540
attagcgtcg gcaatgctta tagctcattt tacgatggtt ggtcacactt cacacaggac 600
ggtacctatg ggtatacaac ccttaatgca atggggaaac tgtacattag gcatgtgaat 660
aggagcagcc ctcatcagat aaccagcacg atcagagtat acttcaaacc caaacacatc 720
aaggcatggg tgccccgacc accacgattg tgcccgtata taaacaaaag ggacgtaaac 780

tttgtagtca cggagataac agactcaagg acttccatca ctgatacacc acacccagaa 840
catagtgtcc tggcaacgca t 861

<210> 62
<211> 879
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 62
ggagacatcg tggaggctgt ggagggagcc atctcgcgag tggcagatac tgttagtagt 60
gggcccagta actctcaagc agtaccagcc ctcacagcag tcgaaacggg tcacacttct 120
caagtcaatc ctagtgacac catgcagacc agacacgtga caaattacca ctcgcggtca 180
gaatccagca tagaaaattt ccttagccgc tctgcttgtg tgtatatggg cgaatacagc 240
acacaagcat cagatgagac caaaaagtac atgtcatgga ccataagccc aaggaggatg 300
gttcaaatgc gcaggaagtt tgagctcttc acttacctgc gttttgatgt ggagattact 360
tttgtaatca ccagcagaca agtcaaggta gggacacaat taggccaaga tgcccccccg 420
ctaactcacc aagtcatgta tataccccca ggaggcccag tacctgattc agttggtgat 480
tacgcatggc agacttccac taaccctagt atcttttgga ccgaaggtaa tgcatcaccc 540
aggatgtcaa taccccttcat tagcataggt aacgcctata gcaactttta tgacgggtgg 600
tcgcattttc accagaatgg cgtctatgga tacaacacgc tgaaccatat ggggcaactg 660
tacgtgcggc atgttaacgg cccttcacca ttaccagtga caagcacagt cagggtctac 720
tttaaaccca aacacgtgaa ggcttgggta ccgagggcac ccaggctatg tcaatatgta 780
aatgcatcca ctgtgaactt cgagccaaca gacatcactg agtcacgcac tgacatcaac 840
catgttccag acaccgtgaa gccagatctc caaacatac 879

<210> 63
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 63
ggggacgtgc acgatgcggt ggttggggcc atgacccgtg ttgcagacac gataagtagt 60
gggccaagca attcagaaag cgtgccagca ttgactgcag ccgagacagg acacacatca 120
caggtagtac cgagtgatac catgcagacc agacatgtgc ggaatttcca cacaagatca 180
gagtcttcaa tagaaaattt catgagtcgc tccgcctgtg tctactatac taagtataag 240
accaaagacc cggacccaac ggagatgtac tctagttgga aggttaccac caggcaagtg 300
gcacaactca ggaggaagat ggagatgttc acttatttgc gctttgacgt agaagtgaca 360
tttgtaataa ctagctcgca agatcagtcc acgagtgttg cacaggacgc acctgttctc 420
actcaccaaa tcatgtacat cccacccgga ggcccggttc ccaaatcagg tagggattac 480
tcatggcaat cctgtactaa cccaagtgtt ttctggactg agggtaatgc accaccacgc 540
atgtgtattc cgttcattag tattggaggg gcatatagtt cattctatga cgggtggtcc 600
cactttaacc aacaaggtcc gtacgggtat aacactctca atgacatggg tcaactgtat 660
tttaggcatg tgaacgaggg tagcccaggg gcggtaacaa gctacatcag aatatacttc 720
aaacctaaac atattagagc atgggtgccc agaccaccta gattgtgtca gtatgagaaa 780

caagggagcg ttgacttcaa ggtgcaggga gtaactgatg ctcgtacctc gctcaccact 840
aca 843

<210> 64
<211> 885
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 64
aatgacccag cacaagccgt gttgagtgcg atcggtcgtg tcgctgacac cgtcgctagc 60
gggccatcga attcagagag agttccagtt ctaaccgctg cggagacagg tcatacctca 120
caggtggttc ccagcgatac cattcagacg cgccacgtcg tcaacttcca cacaagatcg 180
gagtcaacaa ttgaaaattt tatgtgtcgc tccgcctgcg tgtacatcgc ccggtacggt 240
actgaaaagc aaggggaaca aatatccaga tacaccaagt ggaagatcac cactaggcag 300
gtggcgcaac tgcgcaggaa gatggagatg ttcacataca tgcgatttga tttggaaatg 360
acatttgtaa tcacaagctc ccagcgtatg tcaacggcat atgattcaga cacaccagcc 420
ctcacccacc aaataatgta cgtgccacct gggggcccgg agccccgtca ttatgaggat 480
ttcgcctggc agacatccac aaatccaagc atattttgga ccgaaggtaa cgcaccacca 540
cgcttatcaa tcccatttat gagtgtggga aatgcctatt gcaattttta tgatgggtgg 600
tctcactttt cacaaagtgg agtgtatggg tttaccacct taaataacat gggacaactg 660
ttcatgcgcc atgtcaataa gtcaacagcg caccccattg atagtgtggt gcgagtttat 720
tttaaaccaa agcatgttaa ggcgtgggtt ccaagacctc cccggttgtg cccatacatc 780
tatgcaagga acgtggattt tgagccacaa ggtgtcactg aatcaagaga aaagataaca 840
ctagataggg atactcacac ccctatgcgc acatgcgggc cgttc 885

<210> 65
<211> 882
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 65
ggagatgtct gtgaggaagt agagagggct attgtcaggg ttgcagatac tgtcggacgc 60
ggtcctgcta acactgagag tgtaccagcg ctgactgcag ttgaaactgg acacacttca 120
caagttgtac ccggggacac catgcaaacc agacatgtta aaaactttca cacgcggtca 180
gaatcatctg tggaaaattt catgtgcaga gcagcgtgtg tgtattatgt ggattaccac 240
acacaaaatg acagtgagga tgaaaaatat gcatcttgga ttatcaacac gagacaggta 300
gcacagctac gcaggaaaat tgagctgttc acatacacta ggtttgatgt cgaaatcaca 360
ttcgtgatca ccaccacaca gcagcaatcc acagctccca accccgacac tcctctgctg 420
acacaccaaa tcatgtatgt gcccccgggt ggcccagtgc caaatagtgc taccgattat 480
tgttggcaat catccacaaa tcccagtata ttctggaccg agggtagcgc accacccaaa 540
atgtcaatac cctttataag tgtgggaaat gcatacagca gtttttatga tgggtggtca 600
catttcactc aaaacggggt gtacgggttc aacactctga acaatatggg caaattatac 660
ttcaggcacg taaatgacaa caccgtaggg ccatatgtga gcaaagcccg catttatttc 720
aaaccaaagc atgtgcgtgc gtgggttccc aaacctccca ggctctgtga atacaacaat 780

cgagccaacg tgaactttga accacgaggg gttaccgatg ccaggtctag tatcacggcc 840
acaaccgaca cgatcactga gagcacaggg atgcaaacga ct 882

<210> 66
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 66
aatgatccag caactgccat agttagatcg gttgagagag tggctgatac catagcaagt 60
ggacccacta actcagagag agtgccagca ctaaccgccg ttgaaacagg tcacacctca 120
caggtagtcc cgagcgacac catgcaaact aggcatgttg tgaaccatca cattagatca 180
gagtcctcta ttgaaaactt cctgagcagg tccgcctgcg tgtacatcga catgtatggg 240
acaaaagaga atggtgacat caagcgcttc accaactgga gaataaacac acgtcaggtc 300
gtgcagctaa ggcgcaagct ggaaatgttt acatacatta gatttgatgt tgaaatcact 360
tttgtaatca ctagcacaca gggaacaccg actcaaaaga acaaggatac cccagttctt 420
acacaccaaa tcatgtatgt gccaccaggg ggcccaatcc ctgtatctta tgaagattat 480
tcttggcaga cctctacaaa tcctagtgtt ttctggacag aagggaatgc cccagcccgt 540
atgtcaattc ccttcatgag cgtagggaac gcctattgta acttttacga cgggtggtca 600
cacttctcac aatcgggtgt gtatgggttc actacactca ataacatggg tcagttgtac 660
tttcgacacg tgaacaagga cacccttgga ccatacaata gcacggttcg ggtttacttc 720
aaacccaaac atgtgaaggc atgggtaccc agaccaccgc gcctgtgcga ctacgtttac 780
gcacataatg ttgacttcac accaaaaggg gttactgaca gcagggacaa gatcaccctg 840
gaccgtgatg aacacgtgcc gtcagtggtt aaccac 876

<210> 67
<211> 870
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 67
ggagatgatc caccgcattc gatctcaaac acggttgcaa acaccaaccc tagtggtcca 60
accaactcag aaaggatccc agcgctcaca gcagcggaaa ctggtcacac ctcgcaggtg 120
gtcccgagtg ataccgtaca aactcgttgt gtgaaaaact tccacactcg atcggagtca 180
tcaattgaga acttttgtg cagatcagct tgcgcacaca tgtcatcgta tgaggccttc 240
ccaacaacaa cacaagacgg tacacaaagg ttcgccaatt ggacgattag tgtgaaagac 300
atggtgcagt tgaggaggaa atgtgagatg ttcacgtact taagatttga catggaggtg 360
acttttgtga taactagtgt gatcgaaact acaaaaggga aagtaccggc accagcagtc 420
acacaccaag taatgtacat tccaccaggc ggacctattc cagctagcgt tgaaagttat 480
gcctggcaaa catccaccaa cccaagcgtg ttttggacag aagggaatgc tcccccacgc 540
atgtctatac catttatcgg cattggtaat gcctacagca tgttctatga cggatgggcc 600
agtttcagac aatcgggtgg atatggatac agcaccctga accacatggg ccagatattc 660
gtaagacacg tgaatgcaac cataccaaac ttgatcagca cagtcaggat atatttcaag 720
cccaagcacg ttagggcttg gattcctaga ccgcccaggg tgtgtcagta catttacaag 780

gcaaatgtag actacgcagt gtcaaatatc actgaaaagc gagatagtat aagatggaca 840
ccaacaaccg gtccgtcaat gacatcccac 870

<210> 68
<211> 855
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 68
ggtgacgacg caaggactgt tagcgacaca caaaagagcc agccatctaa ctctgagcaa 60
gtgcctgcct taacagcggt tgagactgga cacacctctc aagttgagcc cagtgataca 120
gtacagacac gacatgttgt caactcacac agtaggacag agtcgacaat tgagaatttc 180
tttgggaggg ctgcgtgtgt gagggtgaga gagtactcta tagggcatga tttggcagcg 240
gacgaaacat atgatagctg ggccattaca gtgcgagaca tggtgcagct tcgtaggaag 300
tgtgagatgt tcacatacat gaggtttgac ttggaagtga cgctagtcat caccagctat 360
caagaaccag ggacaatcac cacccaggat atgcccgtcc taacccacca gattatgtat 420
gtgccgccag gaggcccggt cccagccaag gctgacagtt acgcgtggca aacgtcaaca 480
aatcccagta tattctggac cgaaggcaac gctccacctc ggatgtctat cccatacatt 540
ggcatcggca atgcatatag cagcttttat gacgggtggt cgagcttcaa caactcgggt 600
gtgtatggct acacaaccct gaataacatg ggtaaactgt acttcagaca cgtgaacaaa 660
cacagcccaa acactattaa gagcactgtg aggatatatt tcaagcccaa gcacgtccag 720
gcgtgggtcc caagaccacc gcgcttgtgc ccgtatctga ataagaggga tgtcaacttt 780
gaagtgcaac ccgttacgag caagagagac agtattaact gggtgccaca aacaaaccgc 840
caagtgtaca atcat 855

<210> 69
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 69
aatgaaccta gtagtgccat tgagagagca attgtgcgcg tagcagatac tatggccagt 60
gggcctgcaa actcagagca aatccctgcc ctaaccgctg ctgagactgg tcacacctcg 120
caagtggttc ccagcgacac tatgcaaacc cgccatgtat gtaactacca caccagatct 180
gaatcatcga tcgagaactt cctatgcagg gctgcatgtg tctacatagt gagttacaaa 240
acacagggcg acgaacaaac cgacaaatac gctagttggg agatcaacac gcggcaggtg 300
gcacagttaa ggagaaaatt ggaattcttt acttacataa gatttgacat ggaggtaaca 360
tttgtgatca ctggttcaca agacaccagc acacagacta acacggatac gccagtgcta 420
acccatcaaa ttatgtatgt gcctcccggt ggtccagtac cgacatcagc cacagattac 480
agctggcaga catctacaaa tcccagtgtg ttctggacag aagggaatgc gcctccccgt 540
atgtccatac ccttcatgag cataggcaat gcgtatgcta atttctatga tgggtggtcg 600
cactttagcc agtcaggggt gtatggttac accacactca ataatatggg taccctgtat 660
ttcaggcacg tgaacaactc gaccatcggg ccttacacca gtgcagttag gatatatttc 720
aagccaaagc acgtcaaagc gtgggtgcca cgaccgccac ggttgtgcga ttacaaacac 780

aaaaagaacg tagactttac tcccacaggt gtgaccacaa ctagagacaa gataaccttg 840
gacaagggga ctcacgtgcc gagcgtatgg aacaca 876

<210> 70
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 70
aatgaccccg aaggtgcact taataaagca gtgggcaggg tagctgatac tatagctagt 60
gggcccgtca atacagagca aattcctgca ttgacagcag tggagacagg gcatacatct 120
caagtggtac ctagtgacac aatgcaaacc cgacacgtgg tcaacttcca tactagatca 180
gagtcatcgt tacagaactt catggggaga gcggcatgtg tatatatcgc ccactatgcc 240
acagaaaagg ctaatgatga tttggacaga tacactaact gggagatcac aactaggcag 300
gtggcacagt tgaggcgcaa gttggagatg tttacgtata tgagatttga cctcgagatt 360
acattcgtaa tcaccagctc ccagcgtact tccaacaggt atgcgtcaga ctcccccccca 420
ttaacacatc aaataatgta cgtgccgccg gggggtccaa ttcccaaggg ttatgaagac 480
tttgcctggc agacgtccac caacccaagt gtgttttgga ccgaaggtaa cgcccctcct 540
aggatgtcaa taccattcat gagcgttggc aacgcatatt gtaactttta tgatggatgg 600
tcccatttca gtcagagcgg tgtgtacggg tacactacat tgaacaacat ggggcgctta 660
tattttagac atgtaaacaa atcaacagga tacccagtaa atagtgtcgc ccgcgtctat 720
ttcaagccca agcatgtgaa ggcatgggta cctcgcgcgc cacgcttatg tccatatttg 780
tatgctaaaa atgtcaactt tgatgtgcaa ggcgtgaccg agtcccgggg taagatcact 840
ctcgaccgtt cgactcacaa ccccgtgtta accact 876

<210> 71
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 71
aatgaccctg aaggtgcgct caacaaggcg gtgggcagag tggctgatac aatagccagt 60
gggcccgtca acactgagca aattcccgca ttgacagcag tggaaacagg gcacacatct 120
caagtagtac ctagtgatac aatgcaaact cgacacgtgg tcaacttcca caccagatca 180
gaatcatcgt tggagaactt catgggaaga gcagcgtgtg tgtatatcgc tcattatgct 240
acagagaagg ctaatgatga tttagacaga tacaccaact gggaggtcac aaccaggcag 300
gtagcacagt tgaggcgtaa actggagatg ttcacgtaca tgaggtttga cctcgagatc 360
acatttgtaa tcaccagctc ccagcgcact tcaaccaagt atgcgtcaga ttcccccccca 420
ctaacacacc agataatgta tgtaccgccg gggggcccga tccccaaggg ttatgaagat 480
tttgcctggc agacgtccac caacccaagt gtattttgga cggaaggtaa cgcccccccct 540
aggatgtcga taccattcat gagcgttggt aacgcatact gcaactttta cgacggatgg 600
tcccatttca gccagagcgg tgtgtacggg tacactacat tgaacaacat ggggcacttg 660
tatttcagac atgtaaacaa atcaactgca tacccagtta acagtgttgc ccgcgtctac 720
ttcaagccca agcacgtaaa ggcttgggtg cctcgcgcgc cacgcttatg tccatatttg 780

tatgcaaaaa atgtcaattt tgatgtacaa ggtgtgaccg agtctcgggg aaaaatcact 840
cttgatcgat cgactcacaa ccctgtgtca accacg 876

<210> 72
<211> 877
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 72
aacgaccccg aacatgcgtt aaacaacgcc attggtagag tggcagatac gatcgccagt 60
gggccggtga actcggaacg catacctgca ctaaccgcag tggagacagg acacacgtct 120
caagtggtgc caagcgacac catgcaaaca aggcacgtag tcaacatgca tacaagatcc 180
gaatccacca tcgaaaattt catgggaagg gctgcttgtg tatacattgc gcaatacgcc 240
actgataagg ccagtgatga tctggacagg tacaccagct gggagatcac tacgagacag 300
gttgcgcaat tgaggagaaa gctggagctg tttacataca tgaggtatga cttagaagtt 360
acctttgtca ttaccagttc ccagcgcact tcgactacat atgcatcaga ctccccgcca 420
ttgacccacc aaattatgta tgtgcctccc gggggcccta ttcccatagg acacgaagac 480
ttcgcctggc agacttcaac aaaccccagt gtcttttgga ctgaaggaaa tgccccacca 540
cgtatgtcca taccattcat gagtgtgggc aatgcctact gcaattttta cgatgggtgg 600
tcacatttta accagagtgg ggtgtatgga tacactacac taaacaacat gggtcgctta 660
tatttcaggc atgtaaacag atctactgcc tacccagtta atagtgttgc acgtgtttac 720
tttaaaccca aacacgtcaa agcctgggtc ccacgagcac cacgattgtg cccatacttg 780
tatgctaaga acgtgaactt taatgtgcaa ggtgtgactg actcccgaga caagataacc 840
gtagaccgaa ccaaccatgt acgtatgcgc accacag 877

<210> 73
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 73
aacgaccccg aacacgtgtt aaacaatgcc gttggcagag tggcagatac aatcgccagc 60
gggccggtga actcggaacg cgtacctgca ctaactgcag tggagacagg gcatacgtct 120
caagtggtgc caagcgatac tatgcaaaca agacacgtag tcaacatgca cacaagatct 180
gaatccacta tcgaaaattt catgggaagg gctgcttgtg tatacatcgc acaatacgct 240
actgacaaag ccagtgacga tttggatagg tacaccagct gggaaatcac cacgagacag 300
gttgcgcaat tgaggagaaa gttggaaatg ttcacataca tgaggtatga cctggaagtc 360
acctttgtta tcaccagttc ccagcgcacc tcgactacat atgcatcaga ttccccacca 420
ttgactcatc agatcatgta cgtgcctccc gggggcccca ttcctatagg atacgaggac 480
ttcgcctggc aaacatcgac taaccctagt gtcttttgga ctgaaggaaa tgccccacca 540
cgcatgtcca ttccatttat gagtgtgggc aatgcctact gcaattttta cgatgggtgg 600
tcacacttta gccagagtgg ggtgtacgga tacactacac taaataatat gggtcgtctg 660
tatttcaggc atgtaaacaa atctactgcg tacccggtta atagtgttgc acgtatttac 720
ttcaaaccca aacatgttaa agcctgggtc ccgcgagcac cacgactgtg cccatatttg 780

tatgcaagga acgtgaactt taatgtgcaa ggtgtgactg actcccgaga aaagataacc 840
atagaccgaa ccaaccatgt gcccatgcgt aacaca 876

<210> 74
<211> 876
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 74
ggggacacgg aacatgcagt tgagtcagct atctccaggg tagcagatac cattagctca 60
ggtcctagta aactgttgc tataccagcg ctcaccgcgg cagaaacggg ccacacatcg 120
caagtcaccc ccagcgacaa tcttcagacg cgccatgtta agaactatca ctcccgctct 180
gagtcaacta ttgaaaactt cctgtgtaaa tccgcgtgtg tgcatattgc gtcatacaac 240
gcatacggtg atgttggatc agacagtaga tatgatagtt gggagatcaa catcagggaa 300
atggtgcagt taaggaggaa gtgcgaaatg ttcacctatc tcagatttga catggaggtg 360
acatttgtca tcactagcaa gcaagatcaa gggacttcgc tatcacaaga catgccagtg 420
ctaacacatc agatcatgta cgtgccgcca ggcggatccg tgcccactag cgtccagagc 480
tacgcatggc aaacatccac caacccgagc gtgttttgga cagagggcaa tgcccctgct 540
agaatgtcca tcccattcat tagcataggg aatgcataca gcagcttcta cgacgggtgg 600
tcacatttca cccaacaagg tggctatggc tataatacac tgaacaagat gggtaagttg 660
tttgtaaggc atgtgaataa agaaacacca acccatgtga cgagcacgat acgtgtatat 720
tttaaaccaa agcatgttag agcgtgggtg ccaaggccac ctagattgtg cccgtacatc 780
aataaagcgg actgtaactt cgctgttaca ccactcacca aacagcggtt aggaatcaac 840
gatgtcccgc ggcccagcca cacattacat actcat 876

<210> 75
<211> 875
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 75
aacgaccccg caaccgctat tgaaggagca gtccggcgag tggcggacac gatccagagc 60
ggaccgagca attcggagcg ggttccagcg ttaacggccg ttgagacagg tcacacagca 120
caggttaccc cgagtgatac aatgcaaact agacatgtac acaacttcca caccagatcg 180
gagtctagca tcgagaactt cctcagtaga gcagcttgtg tgtacatagg gaaatatagt 240
agcaatgcaa caacacaaga tgaacaatac atgtcatgga caattaatac cagacagatg 300
gtgcagctga gacgcaaatt cgaaatgttc acctacctac gcttcgacgt agaagtcact 360
tttataataa catcgcacca agatcaaggg acacagttca accaggatgc gcccgtaatg 420
tgccaccaaa tcatgtatgt gccacctggt ggcccggtgc ctaagagtgt tgatgacttc 480
acatggcaaa cctctactaa ccctagtgtc ttttggtcag aaggcaatgc accaccgaga 540
atgaccattc cattcattag tatagggaac gcctacagca gcttttatga tggctggtca 600
cacttctctc aaaatggggt ttacgggttt aatgcactca ataacatggg taaactgtat 660
gtgagacaag tgaacctaaa agcccctatg ccagtcagca gtacagttag gatctatttc 720
aaacccaagc atatcaaagc ttgggtaccc agaccaccgc gtctatgtaa gtacctgaag 780

tctgggagtg tcaattttga gcccactgat ttgacagaaa aacggaaatc cagaaagtac 840
atcccaaaaa ctttcagacc agatgtgaga accat 875

<210> 76
<211> 843
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 76
ggtgatgtgc atgatgcagt tgtgggtgcg atgtcgcgcg tcgctgatac agtagcaagt 60
ggccctgcaa actctgagag cgtgcctgct ctcactgcgg tagaaactgg acacacgtca 120
caggtgacac caagtgatac aatgcagacc agacacgtac acaacttcca cacacggtcc 180
gaatcgtcaa tcgagaactt cttaagccgc tctgcatgtg tctattatgc aacgtacaaa 240
acaacagcca gcagacccga agaccaattc gttaggtggt ccatttcata ccgccaggtg 300
gcccaactgc gcaggaaaat ggaaatgttc acctacctgc gctacgatgt ggaggtcact 360
tttgtgatta caagttctca ggacccatcg accaacgtaa gccaggatgc tcctgtactc 420
acacatcagt taatgtacgt accccccggg ggtccagtgc ccaaaaattc aagagactat 480
gcatggcaaa catccaccaa cccgagtgtg ttctggaccg aggggaacgc accaccaagg 540
atatccatcc cctttatcag tgtgggcaac gcatacagtt gcttttatga tggatggtcc 600
cactactcac agacgggggt gtatggttac aacaccttaa acgacatggg ccaattattt 660
gtcaggcacg tgaatgaggc aagcccgggt gcggtgtcaa gtgtagttag gatttacttc 720
aaacccaaac atgtgaaggc atgggtcccg agaccaccac ggttgtgcca atatgttaac 780
gcagcaacgg tgaacttcac tcctgaaggg gtcactaagg cacgtactga tctcatgaca 840
aca 843

<210> 77
<211> 915
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 77
ggaatagaag aaactattga cacagtgatc accaacgctt tacaactgtc tcagcccaaa 60
ccgcagaaac aactcactgc tcaatccacc gcctcatcca gcggagtcaa ttcacaagaa 120
gtgccagcat tgactgctgt ggagacggga gcttctggtc aagccatacc cagcgacgtg 180
attgagacca gacatgtcgt caattacaaa actagatctg aatcaaccct tgagtcattc 240
tttggtagat cagcatgcgt aaccatactg gaagtagaga acttcaatgc cactaccgaa 300
tcggacaaga aaaagcaatt caccacctgg ccaatcacat acaccaacac agtccagttg 360
cgcaggaaat tggaattctt tacatactcc agatttgatc tggaaatgac ttttgtcata 420
actgagaggt accacacaag taatacagga catgctagaa atcaagtgta ccaaataatg 480
tacataccac cgggtgcgcc aaggcccaca gcacgggatg attacacctg gcaaagttca 540
tccaatccat cagtgtttta cacatatggt agcgcgcctc ccagaatgtc tatcccatat 600
gttggcattg ccaatgcata ctcacacttt tatgacgggt ttgcccgagt tcccctgaaa 660
gatgatacaa ctgactccgg tgacactttt tatggattgg tcaccatcaa tgactttgga 720
acattggctg tgagggtggt gaatgagttc aaccctgcaa ggataacatc aaaggtcaga 780

gtttatatga agcccaaaca tgtgaggtgt tggtgtccta ggccaccgcg cgcagtgccc 840
tatcgtggtg aaggggttga tttcaaacaa gattcaatca cgccaataac agcagtcacc 900
aatattaata ccttc 915

<210> 78
<211> 936
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 78
tcaaaccact tacatggagc agaggcagcc tatcaggtgg agagtatcat caaaacagca 60
actgatactg tgaagagtga gattaacgcc gaacttggtg tggtccctag tctaaatgca 120
gttgaaactg gtgcaacttc caacactgaa ccagaagaag ccatacaaac tcgcacagta 180
ataaatcagc atggtgtgtc ggagacgtta gtggagaatt ttcttggtag ggcagcccta 240
gtgtcaaaga aaagttttga atacaagaat catgcctcat ccagcgcagg gacacacaaa 300
aactttttta aatggacaat taatactaag tcttttgtcc agttaagaag aaagctggaa 360
ttattcacat accttaggtt tgatgctgaa atcaccatac tcacaactgt ggcagtaaat 420
ggtaataatg acagcacata catgggtctc cctgacttga cactccaagc aatgtttgta 480
ccaactggtg ctcttactcc aaaggagcag gattcatttc attggcaatc aggcagtaat 540
gctagtgtgt tctttaaaat ttctgatccc ccagctagaa tgactatacc ttttatgtgc 600
atcaactcag catattcagt tttttatgat ggctttgctg gatttgagaa aaatggtcta 660
tatggaataa acccagctga cactattggc aacttgtgtg tcagaatagt gaatgaacat 720
caaccagttg gttttacagt gaccgttagg gtttacatga agcctaaaca tataaaagca 780
tgggctccac gaccaccgcg aaccatgcca tacatgagca ttgctaatgc aaattacaaa 840
ggtagagata cagcaccaaa cacacttaat gccataattg gtaatagagc gagtgtcaca 900
actatgcctc acaacatagt aaccaccggt ccgggt 936

<210> 79
<211> 861
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 79
aatgaccagc acaatggggc gatcgttgcc aacacaacag ctagcggacc ttctaattcg 60
gaaagcatac cggcacttac tgcggctgag actggccaca catcgcaggt tgtccctagc 120
gacaccatcc agacaagaca tgtgaaaaac taccactcgc gttcagagtc caccatagag 180
aacttcctgt gtagatctgc ctgtgtgtac tacaccacgt acaacactca gggcgagcaa 240
gcacatgata aatacgcaag ttggccaatc acgactagaa aagttgccca actgcgcagg 300
aagctggagt tctttaccta cctgcggttt gatctcgaga tcacgttcgt gatcacgagc 360
gcccagatca catccacgaa ccaaaaccag gatgccccag tactcacaca tcaggtgatg 420
tatgtacccc caggggggt ggtaccgcgc agtgtggatg actatagttg gcagacttcc 480
accaatccca gcatcttctg gacagaaggg aacgcacctc ctcgtatgtc aataccattc 540
attagtgtgg gcaacgccta cagcagcttt tacgacgggt ggtcacactt tgaacaaacc 600
ggggtatatg gattcaatac ccttaataat atggggactt tgtacgccag gcacgttaac 660

```
ggtgctagtc ccgggccagt caagagcacc attaggatat atatgaaacc taaacatgtg     720
aaagcgtgga tacctaggcc cccacggttg tgcgactatg tgaaatctgg caacgtcaac     780
tttgaaccaa aaggagtcac cgagagcaga ccatctataa agttagaaaa gacctcaagt     840
gggcacaggc tgacaaccca c                                               861
```

<210> 80
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 80
Met Tyr Val Pro Pro Gly Gly
1 5

<210> 81
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> VARIANT
<222> (0)...(0)
<223> Xaa = any amino acid

<400> 81
Met Tyr Xaa Pro Xaa Gly Ala
1 5

<210> 82
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> VARIANT
<222> (0)...(0)
<223> Xaa = any amino acid

<400> 82
Phe Gly Xaa Gln Ser Gly Ala
1 5

<210> 83
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> VARIANT
<222> (0)...(0)
<223> Xaa = any amino acid

<400> 83
Thr Ala Xaa Glu Thr Gly His
1 5

<210> 84
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<221> VARIANT
<222> (0)...(0)
<223> Xaa = any amino acid

<400> 84
Thr Ala Val Glu Thr Gly Xaa
1 5

<210> 85
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
synthetic construct

<400> 85
Gln Ala Ala Glu Thr Gly Ala
1 5

<210> 86
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; Note =
      synthetic construct

<221> VARIANT
<222> (0)...(0)
<223> Xaa = any amino acid

<400> 86
Met Xaa Xaa Pro Pro Gly Xaa
 1   5

09/937862
410 Rec'd PCT/PTO 28 SEP 2001

DOCKET NO. 14114.0353U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
OBERSTE *et al.*)
Serial No. Unassigned)
Filed: Herewith)
FOR: TYPING OF HUMAN ENTEROVIRUSES)

Group Art Unit: Unassigned

Examiner: Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT (IPEA/EP)
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

September 28, 2001

Sir:

Prior to the issuance of an Office Action pertaining to the above-identified patent application, please enter the following preliminary amendment and consider the following remarks.

IN THE SPECIFICATION

On page 1 of the specification, before the first paragraph, please insert the following:

-- The present application is a 35 U.S.C. § 371 national phase application from, and claims priority to, international application PCT/US00/07828, filed March 24, 2000 (published under PCT Article 21(2) in English), which claims priority to U.S. provisional patent application Serial No. 60/127,464, filed March 31, 1999, which applications are hereby incorporated herein in their entirety by reference.--

REMARKS

The specification is amended herein to update the priority claim for this application. It is believed that no new matter has been added by this amendment, and applicants respectfully request entry of same into the present application.

No fee is believed due; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

Mary L. Miller

Mary L. Miller
Registration No. 39,303

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811
(404) 688-0770

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mailing No. EL491885455US in an envelope addressed to: Assistant Commissioner for Patents, Box PCT (IPEA/EP), Washington, D.C. 20231, on the date shown below.

Everardo McFarlane

9-28-01
Date

TYPING OF HUMAN ENTEROVIRUSES

FIELD OF THE INVENTION

The present invention relates to methods of detecting the presence, and of establishing the serotype, or serovar, of an enterovirus that may be present in a clinical sample or a biological sample, as well as to a kit that includes primers that may be used in the methods. The methods include amplification of viral RNA, and sequencing of the resulting amplicons.

BACKGROUND OF THE INVENTION

Enteroviruses constitute a broad range of pathogens etiologically responsible for a wide range of diseases in humans, as well as in other animals. The genus *Enterovirus* is a member of the family *Picornaviridae*. As the family name indicates, enteroviruses are small RNA viruses; they contain positive single stranded RNA as the genome. Five groups are found within the enteroviruses: coxsackievirus A (CA), coxsackievirus B (CB), echovirus (E), and numbered enteroviruses (EV), as well as poliovirus (PV). There are 66 serotypes currently classified among the human enteroviruses, although two serotypes, E22 and E23, are to be reclassified in a different genus.

The viral genome is shown schematically in Figure 1. The single stranded RNA comprises a 5' nontranslated region (single line), which is followed by an open reading frame coding for a polyprotein precursor of Mr 240-250 x 10^3 Da (boxed portion), followed by a 3' noncoding sequence and a poly (A) tract (single line). In the polyprotein, the sequence of gene products begins 1A, 1B, 1C, 1D, and 2A. 1A through 1D are, respectively, the structural proteins VP4, VP2, VP3, and VP1 of the viral capsid; VP1 is followed in the open reading frame by a nonstructural protein 2A.

The various members of the human enteroviruses cause a wide range of symptoms, syndromes and diseases. These include acute benign pericarditis, acute flaccid paralysis, acute hemorrhagic conjunctivitis, aseptic meningitis, various exanthemas, carditis, croup, encephalitis, enanthema, gastrointestinal disease,

hepatitis, hand-foot-and-mouth disease, various respiratory diseases, myocarditis, neonatal disease including multi-organ failure, pericarditis, pleurodynia, rash, and undifferentiated fever. In general, the syndromes are not correlated with particular enterovirus serotypes, nor does a serotype specifically correlate with a particular disease, although in certain cases serotypes do correlate with particular diseases.

Enteroviruses are responsible for large numbers of infections. There may be between 30 million to 50 million illnesses that are ascribable to enteroviruses each year in the United States (CDC; MMWR 46:748-750; Strikas et al. J. Infect. Dis. 146:346-351 (1986); Rotbart in Human Enterovirus Infections, H. A. Rotbart (ed.) ASM Press, Washington, DC, pp. 401-418 (1995)). After rhinoviruses, enteroviruses are the most common viral infection in humans. Enteroviral infections lead to 30,000 to 50,000 hospitalizations each year for aseptic meningitis, myocarditis, encephalitis, acute hemorrhagic conjunctivitis, nonspecific febrile illnesses, and upper respiratory infections (Melnick, Biologicals 21:305-309 (1993); Morens et al. in Human Enterovirus Infections, H. A. Rotbart (ed.) ASM Press, Washington, DC, pp. 3-23 (1995); Melnick in Fields Virology (B. N. Fields et al. (eds.) 3rd ed., Lippincott-Raven Publishers, Philadelphia, pp. 655-712 (1996)). Enteroviruses are also implicated in acute flaccid paralysis in animal models, as well as in dilated cardiomyopathy. The six serotypes of coxsackie B viruses are implicated in a variety of clinical diseases, such as meningitis, myocarditis and severe neonatal disease. Recently, enterovirus infection has been linked to chronic fatigue syndrome (Clements et al., J. Med. Virol. 45:156-161 (1995)).

Poliovirus is also an enterovirus that infects humans. Three serotypes, PV1, PV2, and PV3 are known. A nonenteroviral picornavirus that also afflicts humans is human rhinovirus (HRV), responsible for many common cold infections; several serotypes have been identified. Additionally, picornaviruses affect mammals other than humans, including viruses such as bovine enterovirus (BEV) and simian picornavirus (SPV).

It is important to identify the serotype of an enterovirus infection in a subject. Knowledge of the serotype can provide useful guidance to a physician in determining

a course of treatment of the disease in the subject. For example, the appropriately identified immune globulin having a sufficient titer may be administered to immunocompromised patients. Furthermore, an antiviral drug such as Pleconaril (Viropharma) may differ in its relative efficacy against different serotypes. Additionally, an understanding of the geographic and chronological development of an enterovirus infection in a population can influence preventive measures among the members of the population to minimize the spread of the disease. Furthermore, it is useful from a broader perspective to track the incidence and distribution of an enterovirus disease from an epidemiological point of view. In earlier practice, it was found that the various serotypes could be grown in different cell culture hosts, and in different animal model hosts. In the animal hosts, furthermore, different symptomology also provided typing information. These classical assays provide ways of distinguishing the serotypes. Nevertheless, some enterovirus serotypes, especially in the coxsackievirus A group, do not grow in cell culture. It has been observed that 25% to 35% of patient specimens are not identified by cell culture for a variety of reasons (Rotbart, 1995). Furthermore, such culturing and classification procedures are costly, time-consuming, subject to experimental variation, and not amenable to repetitive or extensive application in the field.

The serotypes of non-polio enteroviruses have been identified during the past several decades using classical immunological neutralization assays based on a panel of specific antibodies. Application of such a determination to a clinical sample is generally impractical and inconvenient. Although a number of neutralization sites have been localized to the VP1 protein of enteroviral particles, the exact identity of the epitopes responsible for serotype specificity remain unknown; VP2 and VP3 may also contain specific neutralizing epitopes. Serotyping has generally been carried out using intersecting pools of antisera, the Lim and Benyesh-Melnick (LBM) pools, which were originally defined in 1960 (Lim et al., J. Immunol. 84:309-317 (1960)). The antiserum pools currently distributed by the World Health Organization cover 42 serotypes in 8 pools (Melnick et al., Bull. WHO 48:263-268 (1973)). Analysis of the neutralization pattern affords an identification of serotype. (See Rotbart, 1995).

Clearly, this is a cumbersome and painstaking process. Additionally, the supply of the antisera is limited or difficult to maintain. Problems in serotyping more recent isolates have been ascribed to pronounced intratypic antigenic variation (Melnick, Enteroviruses: polioviruses, coxsackie viruses, echoviruses, and newer enteroviruses. In Fields Virology (Fields et al., (Eds.) 3rd Ed., Lippincott-Raven Publishers, Philadelphia, 1996, pp. 655-712; Melnick et al., Bull. W.H.O. 63:453-550 (1985); Wigand et al., Arch. Ges. Virusforsch. 12:29-41 (1962); Wenner et al., Am J. Epidemiol. 85:240-249 (1967); Duncan, Arch. Ges. Virusforsch. 25:93-104 (1968)). This has been explained by pointing out that enteroviruses, being RNA viruses, undergo spontaneous mutation at a very high rate. This can lead to antigen drift, with the potential of producing antigenic variants such that a neutralization assay would produce a false negative result. For example, escape mutants in picornaviruses are discussed in detail in Mateu (Virus Res. 38:1-24 (1995)). For all these reasons there is a need to supplant neutralization assays for serotyping non-polio enteroviruses.

More recently assays based on nucleic acid detection have been developed. Probe hybridization assays directed either to RNA or to cDNA have been used to detect non-polio enteroviruses (Rotbart et al., Mol. Cell. Probes 2:65-73 (1988); Rotbart, J. Clin. Microbiol. 28:438-442 (1990); Chapman et al., J. Clin. Microbiol. 28: 843-850 (1990); Hyypia et al., J. Gen. Virol. 70:3261-3268 (1989); Olive et al. J. Gen. Virol. 71:2141-2147 (1990); Gilmaker et al., J. Med. Virol. 38:54-61 (1992); Yang et al., Virus Res. 24:277-296 (1992); Zoll et al., J. Clin. Microbiol. 30:160-165 (1992); Muir et al., J. Clin. Micro. 31:31-38 (1993); Drebot et al., J. Med. Virol. 44:340-347 (1994); Rotbart et al., J. Clin. Microbiol. 32:2590-2592 (1994)). In the absence of nucleic acid sequence information for the non-polio enteroviruses, most of these probes have targeted the highly conserved 5' non-coding region of the viral genomes. Additionally, RNA probes directed to the VP1 capsid gene have been used on a limited basis to identify some of the CBs and a few closely related CAs (Cova et al., J. Med. Virol. 24:11-18 (1988); Alksnis et al., Mol. Cell. Probes 3:103-108 (1989); Petitjean et al., J. Clin. Microbiol. 28:307-311 (1990)). More recently, oligonucleotides having sequences based on the VP4-VP2 junction have been applied

as diagnostic and epidemiologic tools (Drebot et al., J. Med. Virol. 44:340-347 (1994); Arola et al., J. Clin. Microbiol. 34:313-318 (1996); Kim et al., Arch. Virol. 142:853-860 (1997); Oberste et al., Virus Res. 58:35-43 (1998)). The sequences in these regions, however, do not always correlate with serotype (Kopecka et al., Virus Res. 38:125-136 (1995); Arola et al., J. Clin. Microbiol. 34:313-318 (1996)). Furthermore, sequences of only certain prototpyes were available with which to compare and classify clinical samples (Arola et al., (1996)). A generic probe-based assay for nucleic acids in the presence of chaotropic agents is described in U.S. Patent 5,726,012. An assay for a target nucleic acid sequence wherein two separate probes are hybridized to the same strand of a nucleic acid, and then joined, for example by a polymerase activity, is disclosed in U.S. Patent 5,516,641.

Reverse transcription (RT) coupled with the polymerase chain reaction (PCR) (RT-PCR) has been developed using enterovirus universal primers or broadly selective primers. Such primers are intended to amplify nucleotide regions from a large number of enterovirus serotypes in one diagnosis. One set of primers (Rotbart, J. Clin. Microbiol. 28:438-442 (1990)) has been reported to amplify 60 of the 66 serotypes tested. (Among the nonreactive serotypes, two are atypical enteroviruses and may be reclassified.) A comparison of sequence identities of the various sets of universal primers with serotype sequences is given in Rotbart et al. (1995). Many of the universal primer sets are designed to amplify regions of the 5' untranslated region of the genome (see, for example, Drebot et al. (1994); Diedrich et al., J. Med. Virol. 46:148-152 (1995); Arola et al. (1996); Bailly et al., Virology 215:83-96 (1996); and U.S. Patent 5,075,212 to Rotbart). A comparison of base sequences in coxsackievirus B5 was reported for isolates from three different outbreaks of disease, based on amplicons obtained using primers in the VP1/2A region of the genome (Kopecka et al., (1995)). Variations in sequence occurred even within the same outbreak, and somewhat greater variations were found among isolates from the different outbreaks, and between serotypes. International application WO 98/14611 discloses degenerate primers directed to the VP1 gene, which, when used in certain defined pairs, provide PCR amplification of enterovirus nucleic acids. Use of the specific primer pairs

permits ascertaining whether a sample belongs to an enterovirus serotype, or to a small group of cognate serotypes, based on correlation of the pattern of the presence or absence of an amplicon with priming by the various primer pairs. This method does not rely on obtaining nucleotide sequences for accomplishing the serotyping.

Oberste et al. developed a database of homologous sequences for a portion of the VP2 gene of all 66 human enterovirus serotypes (Virus Res. 58:35-45 (1998a)). They found that the sequences of many antigenic variants failed to cluster with their respective prototype strains as determined by serotyping. This finding suggested that the portion of VP2 examined may not prove to be useful for consistent molecular inference of serotype.

According to Holland et al. (J. Clin. Microbiol. 36:1588-1594 (1998)) neither cell culture growth, nor PCR can successfully type enterovirus infections. They report an alternative typing protocol based on polyacrylamide gel electrophoretic fingerprinting of whole virus radiolabeled proteins. However, the database of viral protein profiles contains data for less than one-third of the known EV serotypes. Therefore its general applicability remains unknown.

In the case of poliovirus, U.S. Patents 5,585,477 and 5,691,134 to Kilpatrick disclose methods and oligonucleotide primers that are specific and sensitive for detecting all genotypes of poliovirus, as well as primers that are specific and sensitive for distinguishing the three serotypes of poliovirus, and methods for detecting poliovirus and/or distinguishing among the serotypes based on the use of the disclosed primers. Additionally WO 98/14611 discloses an extensive set of degenerate oligonucleotide primers for use in detecting the presence or absence of a non-polio enterovirus in a sample and to identify non-polio enterovirus serotypes. The primers are combined in pairs that detect various groupings of serotypes, and several amplification procedures are carried out in order to detect the presence of absence of an amplicon in each case. A pooled grid of the results provides information useful in typing a non-polio enterovirus in a sample.

In summary, immunological methods for serotyping enteroviral infections are cumbersome and time consuming. They rely on an antigen-antibody reaction between

antiserum pools established more than two decades ago, and whose supply may become limited. As explained, for example in Mateu (1995), antigen drift among RNA viruses such as the enteroviruses leads to a high probability that escape mutants will arise, and thereby escape not only serotyping, but perhaps detection as well. A second classical approach, cell culture coupled with whole animal host growth and use of antisera for typing, is extremely cumbersome, expensive, and labor-intensive. Modern molecular biological methods similarly have important deficiencies as currently implemented. Probe assays generally tend to lack sensitivity. Furthermore, a probe directed to a conserved region, such as the 5' non-coding region of the non-polio enteroviruses, lacks specificity, and so cannot be readily applied in typing a viral infection. RT-PCR has been implemented as a generic enteroviral diagnostic assay. In general, these assays fail to implement serotype-specific detection, so that typing is not currently available using RT-PCR. Holland et al. (1998) state that all typing methods in use or then currently under development are limited by virtue of the large number of different enteroviral serotypes, and as a consequence, the need for virus-specific reagents that would discriminate among them.

For these reasons, there remains a need for a typing procedure that avoids the necessity of infecting live animals, animal tissue homogenates, or cell cultures. There further remains a need to implement a nucleic acid-based enteroviral typing procedure that optimizes the specificity required for a typing protocol. There additionally persists a need for a typing procedure that avoids a requirement for a plethora of reagents directed toward the specificity of the various serotypes. There still further remains the need for an enteroviral typing procedure that does not require extended periods of time or complicated procedures to carry out. Thus, there remains a need for an operationally elegant and efficient typing procedure that utilizes the specificity that resides, for example, in the VP1 region. The present invention recognizes these needs, and addresses them.

SUMMARY OF THE INVENTION

As noted above, the determinants of serotype identity are understood to reside primarily in VP1. This amino acid sequence specificity should be reflected in the corresponding VP1 gene sequences. The present invention discloses a method, based on reverse transcription and amplification of a characteristic enteroviral nucleic acid segment, for detecting the presence of an enterovirus in a clinical sample. The method includes the steps of

(i) obtaining a clinical sample from a subject;

(ii) purifying RNA contained in the sample;

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus amplicon is produced whose sequence includes a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome; and

(vi) detecting whether the amplicon is present.

The presence of the amplicon, of course, indicates that an enterovirus is present in the sample.

In important embodiments of the method, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the

VP1 gene. Advantageously, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82. Still more advantageously, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, or an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of these three oligonucleotides are given respectively by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In a further important embodiment of the method of detection, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85. In a further important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among oligonucleotides whose sequences are given by SEQ ID NOs:19, 20, and 21.

In further significant embodiments of the method, the amplification procedure includes a polymerase chain reaction, and the sample is obtained from among whole

blood or a fraction thereof, a bronchial wash, cerobrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal. In still other significant embodiments, the detection is carried out by a procedure chosen from among gel electrophoresis and visualization of amplicons contained in a resulting gel, capillary electrophoresis and detection of the emerging amplicon, probing for the presence of the amplicon using a labeled probe, and labeling a PCR primer employed in the method and detecting the label.

The invention additionally discloses a method for typing an enterovirus in a clinical sample that includes the steps of

(i) obtaining a clinical sample from a subject;

(ii) purifying RNA contained in the sample;

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus sample amplicon

is produced whose sequence includes a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome;

(vi) determining that the sample amplicon is present;

(vii) determining at least a partial nucleotide sequence of the sample amplicon;

(viii) providing a database consisting of prototypical nucleotide sequences, wherein each prototypical sequence is the sequence of a standard amplicon obtained from a member of a set of prototypical enterovirus serotypes by carrying out the procedure of steps (ii) through (v) on each prototypical enterovirus serotype, wherein each prototypical sequence comprises at least a portion of the sequence of the VP1 gene, and wherein the sequence of each prototypical VP1 gene is different from the sequence of every other prototypical VP1 gene in the database;

(ix) comparing the sequence of the sample amplicon with each prototypical sequence in the database; and

(x) identifying the prototypical sequence that has the highest extent of identity to the sequence of the sample amplicon, thereby providing an identified serotype;

wherein the type of the sample is the serotype of the identified serotype.

In important embodiments of this method, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene. More importantly, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82. In significant embodiments of the method, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, at least one oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4 or an oligonucleotide whose sequence contains, at the 3' end thereof,

the sequence given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of the oligonucleotides are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In an additional important embodiment, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85. In a further important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence includes, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more important embodiment, the oligonucleotide mixture contains an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among oligonucleotides whose sequences are given by SEQ ID NOs:19, 20, and 21.

In a further important aspect, the amplification procedure includes a polymerase chain reaction, and the resulting sample amplicon encompasses at least a portion of the nucleotide sequence for the VP1 gene of an enterovirus. The method furthermore importantly provides that the set of prototypical enterovirus serotypes comprises serotypes of coxsackie A viruses, coxsackie B viruses, echoviruses, and numbered enteroviruses. In advantageous aspects of the method, comparing the sequence of the sample amplicon with each sequence in the database employs a sequence alignment and comparison algorithm.

In further important aspects of the method, the sample is chosen from among whole blood or a fraction thereof, a bronchial wash, cerobrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool

extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

The present invention further provides an oligonucleotide containing, at the 3' end thereof, a sequence that hybridizes to a nucleotide sequence encoding an amino acid motif chosen from among the sequences given by SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or an oligonucleotide complementary to any of these oligonucleotides. In an advantageous embodiment, the complete sequence of the oligonucleotide is a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from among SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or is an oligonucleotide complementary to any of them.

In particularly important embodiments, such an oligonucleotide is one whose sequence contains, at the 3' end thereof, a sequence chosen from among the sequences given by SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide whose sequence is complementary to any of these oligonucleotides. In still more important embodiments, the sequence of the oligonucleotide consists of a sequence chosen from among SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide that is complementary to any of them.

The present invention further discloses a mixture of oligonucleotides including at least two oligonucleotides, wherein at least one of the oligonucleotides hybridizes to a sense strand of a double stranded nucleic acid and at least one of the oligonucleotides hybridizes to an antisense strand of the nucleic acid. The nucleic acid to which the oligonucleotides hybridize encodes the VP1 gene of an enterovirus, and the oligonucleotides hybridize to sequences that are highly conserved among the group of enteroviruses. The oligonucleotides, when hybridized to the nucleic acid, are

bound in the correct orientation on their respective strands to direct the synthesis of an amplicon encoding at least a portion of the VP1 protein of enteroviruses when they are employed in an amplification procedure using the nucleic acid.

In important embodiments of the mixture, each oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to the nucleic acid. In still more important embodiments, the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene. Advantageously, at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:80 or SEQ ID NO:81, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding an amino acid motif given by SEQ ID NO:82. Still more advantageously, the mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9. In a highly advantageous embodiment, the sequences of the oligonucleotides are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In an important embodiment, at least one oligonucleotide of the mixture includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide includes, at the 3' end thereof, a sequence that hybridizes to a sequence encoding the amino acid motif given by the sequences of either SEQ ID NO:83, SEQ ID NO:84, or SEQ ID NO:85.

In additional significant embodiments, the oligonucleotide mixture includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more significant

embodiment, the oligonucleotide mixture includes an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

The present invention additionally provides a kit for use in conducting the typing method that includes a mixture of oligonucleotides, the mixture containing an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9. In important embodiments of the kit, the oligonucleotide sequences are given by SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:9.

In additional significant embodiments, the kit includes an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21. In a still more significant embodiment, the oligonucleotide mixture includes an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from among an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of the non-polio enterovirus genome.

Figure 2 illustrates RT-PCR amplification of all enterovirus prototype strains using primer pairs given by SEQ ID NOs:3 and 4, and by SEQ ID NOs: 3 and 9. PCR

products were resolved by 1% agarose gel electrophoresis and visualized by ethidium bromide staining and UV transillumination. Panel A: Coxsackie A viruses, Coxsackie B viruses, and polioviruses amplified with primer pair given by SEQ ID NOs:3 and 4; Panel B: Coxsackie A viruses, Coxsackie B viruses, and polioviruses amplified with primer pair given by SEQ ID NOs: 3 and 9; Panel C: Echoviruses and numbered enteroviruses amplified with primer pair given by SEQ ID NOs: 3 and 4; Panel D: Echoviruses and numbered enteroviruses simplified with primer pair given by SEQ ID NOs: 3 and 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention advantageously provides methods for serotyping enteroviruses obtained from clinical samples. The methods are easily extended to human poliovirus, human picornaviruses such as human rhinovirus, and nonhuman picornaviruses such as bovine enterovirus and simian picornavirus. The procedures are easily and rapidly implemented using common laboratory procedures and instrumentation. They avoid the need for cumbersome, time-consuming and resource-intensive methods such as cell culture and/or host animal infection. They furthermore avoid reliance on prototypical antiserum pools which may fail to identify an enterovirus in a contemporary clinical sample because of antigen drift and escape from immunological reactivity. The methods of the present invention further advantageously permit identifying a serotype as being the most probable serotype even in the case of antigen drift, since nucleotide sequences are matched to provide a most probable serotype match, or, failing a unique match, a set of most probable serotype matches, even in the absence of a high extent of identity.

As used herein, the non-polio enteroviruses refer to the species/subgroups and serotypes, shown in Table 1, that are known in the field at the present time.

Table 1. Non-polio Enterovirus Species/Subgroups and Serotypes.

Species/Subgroup	Serotypes[a]
Coxsackievirus A	CA1 to CA22, CA24
Coxsackievirus B	CB1-CB6
Echovirus	E1-E7, E9, E11-E27, E29-
Enterovirus (Numbered)	EV68-EV71

(a). Serotypes CA-23, E-10, E-28, and EV-72 have been reclassified (Miller, Clin. Infect. Dis. 16:612-613 (1993)). E-8 has been reclassified (Committee on the Enteroviruses, Virology 16:501-504 (1962); Harris et al., J. Infect. Dis. 127:63-68 (1973)).

As used herein, a "clinical sample" or a "clinical isolate" relates to any sample obtained from a subject for use in carrying out the procedures of the present invention. In a principal aspect, the subject is suspected of suffering from a disease or syndrome that is at least partially caused by an enterovirus. The subject may also be an asymptomatic individual considered to be at risk of enterovirus infection. The sample may be a cellular sample such as a tissue sample, for example, a sample of lung tissue obtained as a biopsy or post-mortem, a fluid sample such as blood, saliva, sputum, urine, cerebrospinal fluid, or a swabbed sample obtained by swabbing a mucus membrane surface such as a nasal surface, a pharyngeal surface, a buccal surface, and the like, or it may be obtained from an excretion such as feces, or it may be obtained from other bodily tissues or body fluids commonly used in clinical diagnostic testing. In its broadest sense, a "clinical sample" or a "clinical isolate" as used herein is obtained from a human subject or a non-human mammalian subject, and is directed to suspected symptoms or syndromes ascribable to a picornavirus or enterovirus infection.

As used herein, purification of RNA as a step in the methods of the invention, in particular, as a step leading up to a RT-PCR procedure, relates to releasing RNA

from a latent or inaccessible form in a virion or a cell and allowing the RNA to become freely available. In such a state, it is suitable for effective amplification by reverse transcription and use of the polymerase chain reaction. Releasing RNA may include steps that achieve the disruption of virions containing viral RNA, as well as disruption of cells that may harbor such virions. Purification of RNA is generally carried out under conditions that rigorously and effectively exclude or inhibit any ribonuclease activity that may be present. Additionally, purification of RNA may include steps that achieve at least a partial separation of the RNA dissolved in an aqueous medium from other cellular or viral components, wherein such components may be either particulate or dissolved.

As used herein, "reverse transcription" or "RT" relates to a procedure catalyzed by an enzyme activity, reverse transcriptase, that synthesizes a cDNA from a single stranded RNA molecule, with the use of oligonucleotide primers having free 3'-hydroxyl groups. As used herein the term "polymerase chain reaction" or "PCR" relates to a procedure whereby a limited segment of a nucleic acid molecule, which frequently is a desired or targeted segment, is amplified repetitively to produce a large amount of DNA molecules which consist only of that segment. The procedure depends on repetition of a large number of priming and transcription cycles. In each cycle, two oligonucleotide primers bind to the segment, and define the limits of the segment. A primer-dependent DNA polymerase then transcribes, or replicates, the strands to which the primers have bound. Thus, in each cycle, the number of DNA duplexes is doubled.

As used herein the term "primer" or "oligonucleotide primer" relates to an oligonucleotide having a specific or desired nucleotide sequence which is complementary to a particular sequence on one of the strands of a DNA duplex. When the primer is caused to hybridize to the specific sequence in a DNA duplex to which it is complementary, it may serve as the priming position, or the initiation position, for the action of a primer-dependent DNA polymerase activity. The primer, once hybridized, acts to define the 5' end of the operation of the transcription activity of the polymerase on the duplex. Commonly in PCR, a specific pair of primers is

employed, wherein one of the primers hybridizes to one of the strands and the second primer hybridizes to the complementary strand. The primers hybridize in such an orientation that transcription, which proceeds in the direction from 5'- to 3'-, is in the direction leading from each primer toward the site of hybridization of the other primer. After several rounds of hybridization and transcription the amplified DNA produced is a segment having a defined length whose ends are defined by the sites to which the primers hybridize.

The oligonucleotide primers of the invention are intended for use in a RT-PCR-based amplification of a target segment of a nucleic acid from an enterovirus. Both RT and PCR rely on the action of a DNA polymerase activity to extend the new DNA strands beyond the 3' termini of the primers. Since DNA polymerases extend a chain in the direction from 5' to 3', an oligonucleotide that contains sequences in addition to those nucleotides that hybridize to the target nucleic acid and serve as the primer must have the primer sequence at the 3' end of the oligonucleotide. Additionally, any complements of the oligonucleotides contemplated in the invention must have the sequence complementary to the hybridizing sequence at the 5' end of the molecule such that action of a DNA polymerase will generate a primer oligonucleotide having its complementary sequence at its 3' end.

As used herein the terms "specific to" or "specific for" a target sequence, in relation to a nucleic acid sequence such as an oligonucleotide sequence, relate to a nucleotide sequence that hybridizes, under conditions used in given experimental circumstances, to the target but does not hybridize under those circumstances to sequences that are not target sequences. Nucleotide sequences that are specific for a particular target, such as the enteroviral target sequences that are included in the subject matter of the present invention, are those that include bases all of which are complementary to the corresponding base on the target.

Further as used herein, "specificity" of a nucleic acid sequence for a target sequence also encompasses nucleic acids and oligonucleotides having a small number of nucleotides which may not be complementary to the corresponding nucleotides of the target sequence. Such sequences are still "specific" for the target sequence, as

used herein, as long as the extent of deviation from complementarity remains functionally of no consequence. In particular, such a sequence is "specific" for the target sequence as long as it hybridizes effectively to the target sequence but does not hybridize to any sequence that is not a target sequence, under the conditions used in given experimental circumstances.

As used herein, an "amplicon" relates to a double stranded nucleic acid segment having a defined size and sequence that results from an amplification procedure, such as a PCR procedure. The size of the amplicon is governed by the sites on the two strands of a nucleic acid duplex to which the primers bind. As explained in U.S. Patent 4,683,195, that segment of the product nucleic acid becomes the prevalent product of the amplification procedure after a small number of cycles of amplification.

As used herein, the terms "prototype", "prototypical sequence", "prototypical amplicon", and "prototypical enterovirus serotype" relate, insofar as the root "prototyp-" occurs in each of these terms, to the enterovirus serotypes which were used to establish the classical antisera defined against each serotype. These were originally obtained several decades ago, as described in Lim et al. (1960) and subsequently, for example, in Melnick et al. (Bull. Wld. Hlth. Org. 48:2163-268 (1973)), and Melnick et al. (1985). As used herein, these terms are distinguished from variants of a given prototypical serotype, wherein a variant represents a phenotype resulting from antigenic drift, such as a phenotype that may represent an escape mutant. Such variants may occur in the field among contemporary clinical isolates of enteroviruses.

As used herein, a "motif" relates to a short sequence of amino acid residues that is highly conserved among a family of proteins from different species or variants.

Developing a Database of Nucleotide Sequences Characteristic of the Prototypical Enteroviruses. In order to practice the methods of the present invention, a database of sequences characteristic of the prototypical enteroviruses is needed. In order to prepare such a database, a region of the enteroviral genome is selected that has within its nucleotide sequence sufficient variation among the

different serotypes that the sequence from each serotype may be considered to be unique. In the present invention, the VP1 region of the viral RNA was identified as having the requisite sequence uniqueness from one serotype to another. Among the entries in Table 2, below, direct comparison of results based on VP1 versus those obtained with VP2 for the following variants of the respective serotypes provided evidence that VP1 affords the selectivity required for this invention, whereas VP2 does not. The variants are CA24v strain EH24/70, E4 strain Du Toit, E4 strain Shropshire, E6 strain Charles, E6' strain Cox, E6" strain Burgess, E8 strain Bryson, E9 strain Barty, E11' strain Silva, E30 strain Frater, E30 strain Giles, E30 strain PR-17, E34 strain DN-19, PV1 strain Sabin, PV2 strain Sabin, and PV3 strain Sabin. Once such a region is identified, the nucleotide sequences from this region are determined for each virus among the set of prototypical serotypes. The serotype prototypes of interest in the present invention are listed in Tables 1 and 2; Table 2 includes entries for additional enteroviruses and picornaviruses as well. The viruses may be obtained from publicly available deposits made at the American Type Culture Collection (Rockville, MD).

Table 2. Enterovirus and Picornavirus VP1 Sequences Used in Establishing a Sequence Database

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
CA1	Tompkins	AF081293	23
CA2	Fleetwood	L28146 (a)	
CA3	Olson	AF081294	24
CA4	High Point	AF081295	25
CA6	Swartz	AF081296	26
CA6	Gdula	AF081297	27
CA7	AB-IV	AF061298	28
CA8	Donovan	AF081299	29
CA9	Griggs	D00627 (b)	

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
CA10	Kowalik	AF081300	34
CA11	Belgium-1	AF081300	34
CA12	Texas-12	AF081302	32
CA18	Flores	AF081303	33
CA14	G-14	AF081311	34
CA10	G-9	AF081309	35
CA18	G-10	U05876 (c)	
CA18	G-10	AF081306	36
CA18	G-13	AF081307	37
CA19	8663	AF081309	38
CA20	IH-35	AF081309	38
CA21	Kuykendall	D00538 (d)	
CA22	Chulman	AF081311	40
CA20	Joseph	AF081311	40
CA24v	EH24/70	D90457 (e)	
CB1	Conn-5	M16560 (f)	
CB2	Ohio-1	AF081312	42
CB3	Nancy	M16572 (g)	
CB4	JVB	D00149 (h)	
CB5	Faulkner	X67706 (i)	
CB6	Schmitt	AF081313	40
E1	Farouk	AF081314	44
E2	Cornelis	AF081315	45
E3	Morrisey	AF081316	46
E4	Pesacek	AF081317	47
E4	Du Toit	AF081318	48
E4	Shropshire	AF081319	49
E5	Noyce	AF081320	50
E6	Charles	U16283 (j)	

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
E6	D'Amori	AF081321	51
E6'	Cox	AF081322	52
E6"	Burgess	AF081323	53
E7	Wallace	AF081324	54
E8	Bryson	AF081325	55
E9	Hill	X84981 (k)	
E9	Barty	X92886 (l)	
E11	Gregory	X80059 (m)	
E11'	Silva	AF081326	56
E13	Travis	X79047 (n)	
E14	Del Carmen	AF081327	57
E14	Tow	AF081328	58
E15	CII96-51	AF081329	59
E16	Harrington	X89545 (o)	
E17	CHHE-29	AF081330	60
E18	Metcalf	AF081331	61
E19	Burke	AF081332	62
E20	JV-1	AF081333	63
E21	Farina	AF081334	64
E22	Harris	S45208 (o)	
E23	Williamson	AF055846 (p)	
E24	De Camp	AF081335	65
E25	JV-4	AF081336	66
E26	Coronel	AF081337	67
E27	Bacon	AF081338	68
E29	JV-10	AF081339	69
E30	Bastianni	AF081340	70
E30	Frater	AF081341	71
E30	Giles	AF081342	72

Serotype	Strain	GenBank Accession Number	SEQ ID NO:
E30	PR-17	AF081343	73
E31	Caldwell	AF081344	74
E32	PR-10	AF081345	75
E33	Toluca-3	AF081346	76
E34a	DN-19	AF081347	77
EV68	Fermon	AF08I348	79
EV69	Toluca-1	AF081349	79
EV70	J670/71	D00820 (q)	
EV71	BrCr	U22521 (r)	
PV1	Mahoney	J02281(s)	
PV1	Sabin	V01150 (t)	
PV2	Lansing	M12197 (u)	
PV2	Sabin	X00595 (v)	
PV3	Leon	K01392 (w)	
PV3	Sabin	X00596 (v)	
BEV1	VG-5-27	D00214 (x)	
BEV2a	RM-2	X79369 (y)	
BEV2b	PS-87	X79368 (y)	
HRV3	Unknown	U60874	
PEV9	UKG/410/73	Y14459 (z)	
SVDV	H/3'76	D00435 (h)	
HRV1b	Unknown	D00239(dd)	
HRV2	Unknown	X02316 (aa)	
HRV3	Unknown	U60874	
HRV14	Unknown	K02121, X01087 (bb)	
HRV16	Unknown	L24917(ee)	
HRV89	41467 Gallo	M16248(ff)	
HAV	HM-175	M14707 (cc)	

Notes for Table 2:

PEV, porcine enterovirus; SVDV, swine vesicular disease virus; HRV, human rhinovirus; HAV, hepatitis A virus.

a) Pulli, T., et al., Virology 211:30-38 (1995).
b) Chang, K., et al., J. Gen. Virol. 70:3269-3280 (1989).
c) Poyry, T., et al., Virology 202:982-987 (1994).
d) Hughes, P.J., et al. J. Gen. Virol. 70:2943-2952 (1989).
e) Supanaranond, K., et al., Virus. Genes 6:149-158 (1992).
f) Iizuka, N., et al. Virology 156:64-73 (1987).
g) Lindberg, A. M., et al., Virology 156:50-63 (1987).
h) Jenkins, O., et al., J. Gen. Virol. 68:1835-1848 (1987).
i) Zhang, G., et al., J. Gen. Virol. 74:845-853 (1993).
j) Harris, L.F., et al., J. Infect. Dis. 127:63-68 (1973).
k) Zimmermann, H., et al., Virus Res. 39:311-319 (1995).
l) Zimmermann, H., et al., Virus Genes 12:149-154 (1996).
m) Dahllund, L., et al., Virus Res. 35:215-223 (1995).
n) Kraus, W., et al. J. Virol. 69:5853-5858 (1995).
o) Huttunen, P., et al., J. Gen. Virol. 77:715-725 (1996).
p) Oberste, M.S., et al., Virus. Res. 56:217-223 (1998).
q) Ryan, M.D., et al., J. Gen. Virol. 71:2291-2299 (1990).
r) Brown, B.A., et al., Virus. Res. 39:195-205 (1995).
s) Kitamura, N.B., et al., Nature 291:547-553 (1981); Racaniello, V.R., et al. Proc. Natl. Acad. Sci. USA 78:4887-4891 (1981).
t) Dorner, A.J., et al., J. Virol. 42:1017-1028 (1982); Emini, E. A., et al., J. Virol. 42:194-199 (1982); Nomoto, A., et al. Proc. Natl. Acad. Sci. USA 79:5793-5797 (1982).
u) La Monica, N., et al., J. Virol. 57:515-525 (1986).
v) Toyoda, H., et al. J. Mol. Biol. 174:561-585 (1984).
w) Stanway, G., et al. Proc. Natl. Acad. Sci. USA 81:1539-1543 (1984).
x) Earle, J. A., et al., J. Gen. Virol. 69:253-263 (1988).
y) McNally, R.M., et al., Arch. Virol. 139:287-299 (1994).
z) Peng, J., et al., Unpublished data.
aa) Skern, T., et al., Nucl. Acids Res. 13:2117-2126 (1985).
bb) Callaghan, P.L., et al., Proc. Natl. Acad. Sci USA 82:732-736 (1985); Stenway, G., et al., Nucl. Acids Res. 12:7859-7875 (1984).
cc) Cohen, J. L., et al., J. Virol. 61:50-59 (1987).
dd) Hughes, P.J., et al., J. gen. VFirol. 69:49-58 (1988).
ee) Lee, W.M., et al., Virus Genes 9:177-181 (1995).
ff) Duechler, M., et al., Proc Natl. Acad. Sci. USA 84:2605-2609 (1987).

The virus specimens are used to infect any enterovirus-susceptible cell line in culture, including, by way of nonlimiting example, RD (human rhabdomyoscarcoma) cells, HLF (human embryonic lung fibroblast) cells, LLC-MK_2 (monkey kidney) cells, or BGM (buffalo green monkey kidney) cells; alternatively, a tissue homogenate in tissue culture medium may be prepared from mouse brain after infection of the mouse with the virus. In the case of cell cultures, the culture supernatant is used. In the case of the brain homogenate, the whole homogenate, after growth of the virus, is used. Viral RNA is extracted from the growth media containing the enterovirus prototypes

by any method that releases the RNA from the virion and/or the cell components and provides a purified preparation of the RNA. By way of nonlimiting example, the RNA may be extracted using guanidinium isothiocyanate, such as the single-step isolation by acid guanidinium thiocyanate-phenol-chloroform extraction of Chomczynski et al. (Anal. Biochem. 162:156-159 (1987)). Alternatively, the virion may be disrupted by a suitable detergent in the presence of proteases and/or inhibitors of ribonuclease activity. The RNA released from the virion is isolated or purified, using, for example, methods such as precipitation with an alcohol (e.g., ethyl alcohol or isopropyl alcohol) or banding in a suitable density gradient using an ultracentrifuge.

The purified viral RNA is then subjected to a reverse transcription to prepare a cognate cDNA that encompasses the region of the genome chosen for discriminating between serotypes (i.e., the region encoding VP1). An advantageous way of achieving this is to use a set of random oligonucleotide primers in the reverse transcription, such that certain of the primers in the set will hybridize to the RNA and yield one or more cDNA molecules from the virus encompassing the required serotype-specific nucleotide sequence. Alternatively, gene-specific primers based on a viral RNA-specific sequence from a suitable cDNA may be employed for reverse transcription. Subsequently, the cDNA is amplified using a suitable amplification protocol. By way of nonlimiting example, a polymerase chain reaction (PCR) protocol may be employed for this purpose. PCR is described in operational detail in, for example, "Molecular Cloning: A Laboratory Manual," 2nd ed., Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; "Current Protocols in Molecular Biology," Ausubel et al., John Wiley and Sons, New York 1987 (updated quarterly); and "PCR Protocols: A Guide to Methods and Applications," Innis et al., Academic Press, San Diego, CA 1990; and in U.S. Patents 4,683,195; 4,683,202; 4,965,188; 5,578,467; 5,545,522; and 5,624,833, all of which are incorporated herein by reference.

For the PCR of the cDNA to yield an amplicon containing a sequence from the VP1 region, primers such as those provided in Table 3 (SEQ ID NOs:1-22) may be

employed. In Table 3, nucleotide sequence positions are given relative to the sequence of poliovirus1-Mahoney (Kitamura, N.B., et al., Nature 291:547-553 (1981); Racaniello, V.R., et al. Proc. Natl. Acad. Sci. USA 78:4887-4891 (1981)).

Table 3. Primers Used for PCR Amplification of the VP1 Region of Enteroviruses

Primer	Sequence	Gene	Position	SEQ ID NO
008	GCRTGCAAGAYTTCTCWGT	VP3	2411-2430	1
009	NGCNCCDGAPPTTGNTGSCC	2A	3409-3391	2
011	GCICCIGAYTGITGICCRAA	2A	3408-3389	3
012	ATGTAYGTICCICCIGGIGG	VP1	2951-2970	4
013	GGIGCRTTICCYTGIGTCCA	VP1	3051-3032	5
013	ACRTGICIIGTYTGCATIGT	VP1	2676-2657	6
035	AWITTYTAYGAYGGITGG	VP1	3098-3115	7
036	TAIAIIGTICCCATRTTRTT	VP1	3201-3182	8
046	ATGTAYRTICCIMCIGGIGC	VP1	2951-2970	9
011	GGIGGIGGRTCIGTJAKYTT	VP1	3054-3035	16
045	GAIGARAAYCTIATIGARAC	VP1	2648-2667	17
046	CCCATIAKRTCIATRTCCC	VP1	2820-2801	12
050	GTRCTYACIAIIAGRTCYCT	2A	3513-3494	13
051	TSAARYTGTGCAARGACAC	VP3	2429-2448	14
052	STGYCCAGATTCAGTGT	VP3	2413-2430	15
053	GGNACNCAYRTNATHTGGGA	VP3	2216-2235	16
054	GCCITRTTITGRTGICCRAA	2A	3408-3389	17
055	GGIACICAYRTIRTITGGGA	VP3	2216-2235	18
187	ACIGCIGYIGARACIGGNCA	VP1	2612-2631	19
188	ACIGCIGTIGARACIGGNG	VP1	2612-2630	20
189	CARGCIGCIGARACIGGNGC	VP1	2612-2631	21
222	CICCIGGIGGIAYRWACAT	VP1	2969-2951	22

These primers were designed to amplify a broad range of cDNA fragments drawn from the set of enteroviruses (see Example 2). The primers of SEQ ID NOs:1-22 were designed based on information available regarding known sequences of non-polio enteroviruses, as well as sequences in the VP1 region obtained as part of the development of the present invention (see Example 1; see Table 2 for GenBank accession numbers of the sequences). Additional information used to design the primers of SEQ ID NOs:1-22, especially the primers of SEQ ID NOs:19-22, was obtained from known sequences of other members of the *Picornaviridae* family, as provided in Table 2.

The amplicons obtained from the PCR protocol applied to each prototype virus are sequenced to obtain the nucleotide sequence in each. Procedures that may be used for sequencing include the methods of Maxam and Gilbert (Meth. Enzymol. 65, 499-566 (1980)) and Sanger et al., (Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977)) (see also Sambrook et al., (1989)). The method of Maxam and Gilbert involves random chemical degradation reactions carried out on a nucleic acid labeled at one end. Each of four separate degradation reactions is specific for a different one of the four bases in the nucleic acid. The method of Sanger et al. involves use of a different 2',3'-dideoxynucleotide chain terminator in each of four template-driven DNA polymerase reactions. The Sanger method is readily implemented in automated sequencing instruments, such as those of PE-Biosystems, Foster City, CA. The VP1 sequences that were obtained with the above procedures were incorporated into the non-polio enterovirus database of the present invention (see Table 2).

Typing of Clinical Isolates Obtained in the Field. A clinical sample is obtained from a subject suspected of harboring an enterovirus. Any suitable clinical specimen may be used for this purpose. Commonly, and by way of nonlimiting example, such a sample may be whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal

cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, or tissue from an experimentally infected animal.

Viral RNA may be isolated from a clinical sample either directly or after inoculating a cell culture with the clinical sample and cultivating a larger virus population. Direct isolation is rapid but may result in low virus titer, whereas inoculation and cell culture will provide a higher titer but may take several days.

In order to obtain amplicons from viral RNA, the RNAs from the virus isolates are treated with a reverse transcriptase primer preparation that contains a random oligonucleotide RT primer, such as a library of random hexanucleotides. The resulting cDNA is amplified in a PCR procedure using a mixture of oligonucleotide primers that hybridize to motifs that are highly conserved throughout the enteroviruses, or more generally, motifs that are highly conserved among the picornaviruses. As used herein, the notion of hybridizing specifically to a highly conserved region encoding a highly conserved amino acid motif relates to identifying at least two nucleotide sequences in the viral genomes which display minimal variation across both the complete spectrum of prototypical enterovirus serotypes, as well as the variants that may be present in clinical samples at any given time. Thus, at least two relatively constant amino acid sequences, or motifs, encoded by these nucleotide sequences, occur phenotypically in all or most of the viruses of the enteroviral species and variants, and the corresponding coding sequences in the nucleic acid are likewise relatively constant across the prototypes and variants. Such conserved or invariant sequences, or motifs, are required in order that a single pair of oligonucleotide primers, or as small a set of such primers as is practical, suffices to prime the amplification of all or the maximum possible number of prototypical viruses and all or the maximum number of viral variants infecting the population at any given time.

In important embodiments of the invention, the primers used are a mixture of oligonucleotides whose use in a PCR amplification provides an amplicon encompassing most or all of the VP1 gene. By way of nonlimiting example, such a mixture may include an oligonucleotide chosen from among an oligonucleotide whose

sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:4, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:9, and a mixture thereof, as well as an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:3 (see Table 3); in particularly important embodiments the oligonucleotides employed according to the above mixtures are primer 011 (SEQ ID NO:3), primer 012 (SEQ ID NO:4), and primer 040 (SEQ ID NO:9). The use of either or both of the primers (012, SEQ ID NO:4 and 040, SEQ ID NO:9) provides specific hybridization to target sequences in the 5' region of the VP1 gene of most or all of the non-polio enteroviruses. The third primer, 011 (SEQ ID NO:3), specifically hybridizes to a target sequence in the 2A region of most or all the non-polio enteroviruses. Each of the primers is disclosed in PCT application WO 98/14611, which is incorporated herein by reference.

More generally, primer sets that include a mixture of oligonucleotides that contain the sequences given by SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22 may be employed in amplifying a broad range of picornaviruses. Specifically, oligonucleotides chosen from among an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:20, an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:21, and mixtures thereof, may be combined with an oligonucleotide whose sequence contains, at the 3' end thereof, the sequence given by SEQ ID NO:22 (see Table 3) for use in the present method. Advantageously, the oligonucleotides included in the above mixtures are primer 187 (SEQ ID NO:19), primer 188 (SEQ ID NO:20), primer 189 (SEQ ID NO:21), and primer 222 (SEQ ID NO:22).

Using the mixtures of oligonucleotide primers set forth in the preceding paragraphs leads to preparation of the enteroviral PCR amplicons according to the method of this invention . The amplicons are then either detected or isolated for sequence analysis. They may be isolated by any of a variety of amplicon purification procedures that serve to provide a purified preparation of the amplicon. These

include, by way of nonlimiting example, gel electrophoresis coupled with visualization using a fluorescent dye and extraction of the detected amplicon from the gel, and extraction from the amplification solution using an immobilized derivative of one or more of the PCR primers to bind a strand of the amplicon after it has been denatured. The purified amplicons may be sequenced using conventional sequencing techniques or procedures.

The nucleotide sequence obtained for the amplicon derived from a particular clinical sample of an enterovirus is then matched with the sequences in the database of prototypical sequences describing the known serotypes of enteroviruses. The sequence matching may be carried out by any suitable sequence matching algorithm designed to determine the extent of identity or similarity between a query sequence in its entirety and a standard or reference sequence. By way of nonlimiting example, such an algorithm may be that of Needleman and Wunsch (J. Mol. Biol. 48:443-453 (1970) implemented in the program Gap in the Wisconsin Sequence Analysis Package, version 9.1), and the like. Such algorithms provide a result that the query sequence most resembles a particular one, and (in most cases) only one, of the reference sequences drawn from the database. According to the present method, the serotype of the enterovirus in the clinical sample is the serotype of the sequence from the database identified as most closely resembling the sequence of the sample.

Numerous advantages result upon implementation of the present invention. Typing of an enterovirus in a clinical sample may be done avoiding the necessity of culturing the sample in a cell culture or in a whole animal host (e.g., mouse). Such procedures are cumbersome, labor-intensive and resource-intensive, and pose dangers of infection to the workers conducting the assay. The typing likewise avoids the necessity of conducting a standardized serotyping assay. Serotyping is labor-intensive, and requires the availability of the antiserum pools that are specific or selective for the various enterovirus serotypes. Furthermore, serotyping using these procedures is not very effective because numerous variants and escape mutants in field samples of enteroviruses avoid detection and provide, therefore, a false negative result. The present invention additionally avoids the disadvantages of known PCR

amplification procedures employed with non-polio enteroviruses, which are largely based on the conserved 5' untranslated region of the non-polio enterovirus genome, and thereby lack a means for typing the samples found.

In contrast, the present invention provides the only PCR-based means for typing a clinical sample of an enterovirus available at the present time. The procedure is easy to carry out and provides an unambiguous, and accurate, typing of a clinical sample in a large fraction of test cases that were also typed by standard serotype pools. Typing of cases of enterovirus-caused diseases or syndromes permits an appropriate therapy to be chosen in suitable cases. Such therapy should lead to amelioration of the severity of the disease or syndrome and, hopefully, a complete recovery. Typing furthermore provides important public health and epidemiological information that could lead to protective and/or preventive measures being taken among a population at risk of contracting such a disease or syndrome.

The following examples are intended to illustrate the invention and not to limit it.

<u>Example 1. Establishing a Database of Sequences Corresponding to Standard Non-polio Enterovirus Serotypes</u>. The viruses used for sequence analysis are listed in Table 2, above. The prototypical virus samples were obtained from the American Type Culture Collection. The viruses were propagated in RD cells, HLF cells, LLC-MK_2 cells, or primary monkey kidney cells using Eagle's MEM supplemented with 2% fetal bovine serum or by intracerebral inoculation of newborn mice (see Grandien, M., et al., "Enteroviruses and Reoviruses", in Diagnostic procedures for viral, rickettsial, and chlamydial infections, 6th Ed. (Schmidt, N.J., et al., eds.) 1989, Amer. Public Health Assoc., Washington, DC, pp. 513-578) . The isolation of the viral RNA, and the RT-PCR amplification was conducted as described by Oberste et al. (Am. J. Trop. Med. Hyg. 58:41-46 (1998b)). In summary, in this procedure, viral RNA was extracted from infected cell culture supernatants, or from 10% infected mouse brain homogenate with Trizol LS™ (Life Technologies, Inc., Gaithersburg, MD), and cDNA was obtained by use of a set of random hexanucleotide primers (Boehringer Mannheim Biochemicals, Indianapolis, IN), and a SuperScript™

preamplification kit (Life Technologies, Inc.). Reverse transcription was performed in a solution containing 20 mM Tris chloride pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 M dithiothreitol, 0.5 mM each of dATP, dCTP, dGTP, and TTP, 0.8 μM random hexamer primer, 5 μL RNA, and 10 U SuperScript II™ reverse transcriptase (Life Technologies, Inc.). The reaction proceeded for 1 h at 42°C.

The resulting cDNAs were amplified by PCR using primers for VP3 and 2A shown in Table 3 (SEQ ID NOs:1-18), in a reaction containing 20 mm Tris chloride pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and TTP, 1 μM sense-orientation primer, 1 μM antisense-orientation primer 1 μL cDNA from the reverse transcription step, above, and 1.25 U *Thermus aquaticus* DNA polymerase (Life Technologies, Inc.). The reaction was incubated at 94°C for 3 min, then followed by 35 cycles of 94°C for 30 s, 42°C for 30 s, and 72°C for 30 s, followed by incubation at 72°C for 5 min. The specific primer pairs used differed from one virus to another in order to obtain satisfactory yields of the amplicons. For some viruses, VP1 was amplified as two overlapping fragments with internal VP1 primers as well as the VP3 and 2A primers. The PCR products were gel isolated and purified in preparation for sequencing with the QIAquick™ gel extraction kit (QIAGEN, Inc., Santa Clarita, CA), in which DNA is selectively adsorbed to a silica gel membrane at pH below 7.5 at high salt concentration. The impurities are separated from the membrane, then the DNA is eluted therefrom using Tris buffer or water. Sequencing was carried out on an automated DNA sequencer (Applied Biosystems Division, Perkin Elmer, Inc., Foster City, CA) using 2',3'-dideoxynucleotide chain terminators (Sanger et al. (1977)) that carried fluorescent labels.

Complete VP1 PCR products of viruses for which VP1 primers were not available were obtained by cloning the viral cDNA into the plasmid pGEM-T (Promega Corp., Madison, WI). Nested-deletion subclones were constructed from the resulting plasmid with an Erase-a-Base™ kit (Promega Corp.). In this procedure, the plasmid is first digested with a restriction nuclease providing either a blunt end or a 5' overhang. The opened plasmid is then digested with a 3'-5' exonuclease, *E. coli* exonuclease III, to remove plasmid sequences unrelated to the viral VP1 gene. The

extended 5' overhang is then removed using S1 nuclease, and the plasmid is resealed by first repairing the ends with DNA polymerase, then ligating with DNA ligase. The resulting shortened plasmid is propagated in a suitable host to provide larger amounts of the plasmid, including the VP1 sequence. For each virus, at least two independent clones were sequenced by automated methods as described above.

Using these procedures, complete VP1 nucleotide sequences were determined for 57 human non-polio enterovirus strains for which VP1 sequences had not previously been determined. These are summarized in Table 2, which shows both the GenBank accession numbers (numbers AF081293 to AF081349) and the corresponding SEQ ID NOs, 23-79. Forty-seven of the strains were prototype strains for recognized human enterovirus serotypes (Melnick (1996). The other ten sequenced strains were well-characterized antigenic variants which, while antigenically distinct from their respective prototype strains, were similar enough to them to have been considered to be the same serotype (Committee on Enteroviruses of the National Foundation for Infantile Paralysis, Am. J. Public Health 47:1556-1566 (1957); Melnick (1996)). Combined with the 21 previously available complete enterovirus VP1 sequences, of which 19 are prototypes and 2 are variants, the database constructed for use in the present method includes 66 prototype VP1 sequences and 16 variants or other enteroviruses, including the three poliovirus Sabin strains and the Barty variant of E9.

The boundaries of the newly sequenced VP1 genes were predicted by comparison of the nucleotide and deduced amino acid sequences with those of previously characterized enteroviruses. Human enterovirus VP1 sequences varied in length from 834 to 951 nucleotides (278 to 317 amino acid residues). The CB group has the shortest predicted VP1 amino acid sequences (278 to 298 residues), while EV68 and EV70 had the longest ones (312 and 317 residues, respectively).

Each of the enterovirus VP1 sequences developed in this work is characteristic of the serotype from which it arises, and differs from the sequence of every other serotype. For this reason, the VP1 sequences can be used as markers for the prototypical serotypes of the non-polio enteroviruses. The 66 prototype and 16

variant sequences identified above are used in the method of the present invention to form the content of a database for use in typing an enterovirus obtained in a clinical sample.

Example 2. Design of Non-Polio Enterovirus PCR Primers and Assessment of the Breadth of Their Specificity.

Design of PCR primers. Since the VP1 sequence was found to correlate with serotype (Example 1), this region was targeted for development of sequence-based molecular diagnostics, namely, generic PCR primers to amplify and sequence a portion of the VP1 gene. Degenerate deoxyinosine-containing PCR primers were designed which specifically recognize regions within or near the termini of the VP1 gene of non-polio enteroviruses. Primers with the broadest specificity within the non-polio enterovirus genus were chosen by searching for regions in the genome that encode amino acid motifs within VP1 and those immediately C-terminal to VP1, in 2A, that are the most conserved across the prototypes. (Echoviruses E22 and E23 were excluded, because it is likely that they will be reclassified as members of a new Picornavirus genus, *Parechovirus* (Mayo et al., J. Gen. Virol. 79:649-657 (1997)). The motif MYVPPG (Met-Tyr-Val-Pro-Pro-Gly) was present in the deduced VP1 amino acid sequences of 44 enterovirus prototype strains whose nucleotide sequences are provided in Example 1. Thirteen prototypes had Ile substituted for Val and CA7 contained Ala instead of Val. CA12, CA14, and EV71 contain the motif, MFVPPG (Met-Phe-Val-Pro-Pro-Gly). In EV68 and 70, a slightly different motif was present, MYVPTG (Met-Tyr-Val-Pro-Thr-Gly). For viruses in the CB-like phylogenetic group the M(Y/F)(V/I)PPG motif is followed by Gly, whereas in all other enteroviruses, the motif is followed by Ala (A). To account for differences between the virus groups and for codon degeneracy, two different inosine-containing primers were designed to anneal to this region. Primer 012 (ATGTAYGTICCICCIGGIGG) is based on the amino acid sequence, MYVPPGG (SEQ ID NO:80). Primer 040 (ATGTAYRTICCIMCIGGIGC) is based on the amino acid sequence, MY(V/I)P(P/T)GA (SEQ ID NO:81). The selectivity of these two primers is

primarily due to the first position at the 3' end of each primer (i.e., in primer 012, the base at the 3' end is G, and in primer 040, the base at the 3' end is C) (see Table 3.) In addition, primer 040 contains increased degeneracy at positions 8 and 14 from the 3' end of the primer in order to detect those viruses which encode an isoleucine (position 8) or a threonine (position 14) in these positions. For PCR, primers 012 and 040 were each paired with primer 011 (GCICCIGAYTGITGICCRAA), which corresponds to the amino acid motif FG(Q/H)QSGA (Phe-Gly-(Gln/His)-Gln-Ser-Gly-Ala; SEQ ID NO:82), present near the 5' end of the 2A gene and which is conserved among most enteroviruses for which the 2A sequence is available.

Specificity of PCR Primers. To assess the breadth of specificity and thereby the general applicability of the 012/011 and 040/011 primer pairs, both pairs were tested in RT-PCR reactions with template RNA derived from each of the human non-polio enterovirus prototype strains (see Figure 2). Primer pair 012/011 amplified 23 of 30 echovirus prototypes (Figure 2C), as well as CA2, CA7, CA9, CA11, CB1, CB2, CB3, CB6, and PV1 (Poliovirus 1) (Figure 2A). Primer pair 040/011 amplified 14 of 23 CA prototypes and PV1 (Figure 2B), as well as E2, E6, E14, E16, E18, E19, E20, E24, E25, E27, E30, and E31 (Figure 2D). Twenty-two prototypes were not amplified by either primer pair (CA10, CA13, CA15, CA16, CA20, CA21, CA22, CB4, CB5, E1, E7, E9, E21, E22, E23, E32, EV68, EV 69, EV70, EV71, as well as PV2 and PV3, where PV signifies poliovirus).

Example 3. Typing of Clinical Isolates Obtained in the Field.

Viruses. Fifty-one virus isolates of 24 different serotypes were chosen from those processed in the inventors' laboratory at the Centers for Disease Control and Prevention (CDC) during the period 1991-1998 for routine non-polio enterovirus reference testing. The viruses were from 19 different states in the United States and two other countries, and were chosen to be representative of the serotypes in the collection for the period surveyed. To avoid the effects of sampling bias in the interpretation of sequence comparisons, no more than four isolates of any given

serotype were chosen for sequencing. The isolates included examples of coxsackievirus A, coxsackievirus B, echovirus, and numbered enteroviruses.

Virus isolation and neutralization. The virus strains were isolated from a wide range of clinical specimens, including blood (n=1), cerebrospinal fluid (n=7), conjunctival swab (n=1), "lesion" (n=1), postmortem lung (n=1), nasopharyngeal swab (n=2), sputum (n=1), stool (n=18), throat swab (n=8), and tissue not specified (n=11). Forty-four of the 51 strains were originally isolated by the submitting laboratory, most of which were state public health laboratories in the United States. The remaining seven strains were isolated from original stool specimens at CDC. All isolates were typed antigenically using WHO-standard antiserum pools (Melnick et al., 1973), supplemented with additional pooled and monospecific antisera such that all human enterovirus serotypes, as well as antigenic variants of E4, E6, E11, and E30, could be identified (P. Feorino, personal communication to the inventors).

RNA extraction and RT-PCR. Viral RNA was extracted from infected cell culture supernatant using the QIAamp™ Viral RNA Kit (QIAGEN, Inc.). Reverse-transcription polymerase chain reaction (RT-PCR) was carried out as described previously (Oberste et al., (1998a,b)). From each viral cDNA, an amplicon of approximately 450 bp, encompassing the 3' half of VP1 and the 5' end of 2A, was amplified by PCR using the primers 012/011 or 040/011 (Table 3). Primer specificity was tested by PCR amplification of the prototype strain of each human enterovirus serotype with both primer pairs. Amplification products were visualized by agarose gel electrophoresis and ethidium bromide staining. PCR products from clinical isolates were gel-isolated and purified for sequencing using the QIAquick™ Gel Extraction Kit (QIAGEN, Inc.) and sequenced on an automated DNA sequencer using fluorescent dideoxy-chain terminators as in Example 1 (Applied Biosystems Division, Perkin Elmer, Inc.). The sequences obtained for the clinical samples were deposited in the GenBank sequence database (Accession Numbers AF081595-AF081645).

Sequence analysis. The sequences were compared to the enterovirus VP1 sequence database developed in Example 1 by sequential pairwise alignment of the query sequence with each sequence in the database, using the algorithm of Needleman

and Wunsch (1970), implemented in the program Gap (Wisconsin Sequence Analysis Package, version 9.1). The results of the pairwise comparisons were compiled and sorted in descending order by percent identity with the query sequence.

PCR-amplification of clinical isolates. In order to establish the utility of using viral sequence analysis as an enterovirus typing tool, typing by partial sequencing of VP1 was compared with the conventional serological typing method using 52 clinical isolates typed in the inventors' laboratory from 1991 to 1997. Partial VP1 sequences relate to obtaining sequences in a region of approximately 400 nucleotides at the 3' end of the VP1 gene. Despite the failure of primer pair 012/011 to amplify the E7, E9, E21, CB4 and CB5 prototype strains (see Example 2), 012/011 successfully amplified recent clinical isolates of each these serotypes. Likewise, primer pair 040/011 amplified recent isolates of CA16, CA21, and EV71, but not the prototype strains of these serotypes (see Example 2). Taken together, these two primer pairs failed to amplify only one clinical isolate of the 52 tested, a 1993 EV6 isolate from Texas (TX93-1673). The presence of amplifiable RNA in the latter specimen was confirmed by amplification of 5'-specific sequences by pan-enterovirus primers (data not shown). For the other 51 isolates, a VP1-specific fragment was amplified from purified RNA by RT-PCR using primer pairs 012/011 or 040/011. In most cases, only one of the two primer pairs produced an amplicon of the expected size (data not shown).

Typing of clinical isolates by nucleotide sequence analysis. The PCR products were gel isolated and sequenced. The sequences were compared to the complete enterovirus VP1 database developed in Example 1 by pairwise alignment of the isolate sequence to each sequence in the database using the program Gap. These comparisons produced, for each clinical isolate, a set of values of the percent identity giving the extent of identity between the sequence of the given clinical isolate and each of the prototype sequences in the database. Typing was obtained as that prototype whose extent of identity to the clinical sample was the highest of all the prototypes. In general, as implemented in this study, if the highest global identity is >75%, the clinical sample and the prototype are of the same serotype. If the highest score is 70%-75%, the identification is presumptive and should be confirmed by

neutralization using monospecific antisera specific for each of the four highest scoring prototypes. If the highest score is <70%, the clinical sample is considered to be of no known serotype; for example, it may be from a picornavirus for which a sequence is not yet available, or it may be a new enterovirus serotype. For each clinical isolate, the matches with the highest and second highest pairwise identity score were identified. Table 4 shows the serotype as obtained from the classical neutralization test, as well as the types of the highest and next highest scoring prototypes obtained in this way (with entries giving the extent of identity of both the nucleotide sequences (nt) and the translated amino acid sequences(aa)). Strains in Table 4 are identified by U.S. state (two letter code) or country (three letter code) of origin, year of isolation, and lab identifier number. For example, WA91-0374 indicates that the strain was isolated in the state of Washington in 1991 and the lab sample number was 0374. The abbreviations DOR and PER in Table 4 designate the Dominican Republic and Peru, respectively.

Table 4. **Correspondence Between Typing by Sequence and by Neutralization.**

Strain	Neut. Type	Highest Scoring Prototype			Second Highest Scoring Prototype(s)			
		Type	nt (%)	aa (%)	Type	nt (%)	Type	aa (%)
WA91-0374	E6	E6	83.3	95.6	E1	69.7	E29	74.3
OR91-1426	E30	L30	85.8	92.9	E21	69.5	E21	81.7
CT92-1465	E16	E16	81.4	93.6	E5	72.2	E5	78.6
FL92-1512	CB2	CB2	86.5	98.5	CB4	68.3	CB4	75.2
WA92-1516	E11'	E11	77.1	90.1	E11	72.9	E19	83.0
NC92-1612	E9	E9	77.8	94.6	E17	70.2	E16	72.9
GA92-1616	E11	E11	77.6	89.4	E19	72.2	E19	82.3
TX92-1647	CA14	CA14	86.8	91.1	CA7	63.4	CA7	67.9
MD92-1649	E25	E25	77.1	91.5	E1	68.5	E21	77.6
DOR93-1657	CA24v	CA24	77.4	92.8	CA20	67.6	CA17	75.9
FL93-1763	E11'	E11	78.5	90.1	E19	72.6	E19	83.0
GA93-1763	CA9	CA9	93.8	95.3	E4	68.6	E4	70.8
GA93-1765	E7	E7	79.7	95.7	E32	68.8	E32	77.1
M093-1808	E25	E25	77.6	91.5	E33	67.5	E21	76.9
ME93-1814	CB5	CB5	95.2	98.5	CB1	71.3	CB1	77.7
NM93-1816	CB3	CB3	90.3	97.7	CB6	69.9	CB1	81.5
OR93-1817	E25	E25	77.9	91.5	E1	68.5	E21	76.9
WA93-1821	E4	E4	81.1	96.1	E1	73.1	E1	80.9
MN94-1828	E25	E25	76.9	92.2	E29	67.9	E21	77.6
WA94-1849	E3	E3	79.6	93.0	E7	68.2	E12	80.0
AR94-1884	E30	E30	96.0	93.6	E21	70.0	E21	82.4
GA93-2460	CB5	CB5	95.8	93.5	CB1	70.8	CB1	77.7
GA93-1892	E30	E30	85.5	93.6	E21	69.5	E21	83.4
GA93-1994	E7	E7	79.7	95.7	E32	69.1	E32	77.1
NM94-1919	EV71	EV71	80.6	93.4	CA16	66.9	CA16	76.6
AZ94-1925	CA14	CA14	86.5	97.0	CA7	63.8	CA7	68.2
RI94-1959	E21	E21	78.3	93.7	E30	69.6	E30	80.0
CT94-2006	EV71	EV71	80.3	93.4	CA16	66.0	CA16	76.6

Strain	Neut. Type	Highest Scoring Prototype			Second Highest Scoring Prototype(s)			
		Type	nt (%)	aa (%)	Type	nt (%)	Type	aa (%)
MD95-2037	EV71	EV71	79.9	92.7	CA16	67.0	CA16	76.6
AZ94-2060	CA21	CA21	90.9	98.6	CA24	68.7	CA24	75.5
PA94-5753	CA16	CA16	77.9	94.7	EV71	68.7	EV71	83.0
NM95-2070	E6	E6	76.8	94.1	E29	68.1	E29	75.5
TX95-2089	E13	E13	72.4	88.7	EV69	71.5	EV69	93.0
GA95-2093	CA21	CA21	91.4	98.6	CA24	67.5	CA24	75.5
GA95-2095	CA16	CA16	77.9	94.9	EV71	69.4	EV71	77.4
NC95-2135	CB2	CB2	83.2	99.2	CB4	68.3	CB4	76.2
AR95-2139	E9	E9	75.7	92.8	E17	70.0	E1	71.8
TX95-2147	CA16	CA16	76.5	94.9	EV71	70.4	EV71	77.4
VA95-2154	E11'	E11	78.3	90.8	E19	71.7	E19	83.7
WT95-7151	E9	E9	75.7	93.5	E17	69.4	E16	71.4
VA95-2157	E30	E30	85.3	92.1	E21	70.0	E21	82.1
GA96-2175	CA9	CA9	81.5	92.6	E19	68.4	E11	72.3
CT96-2181	E5	E5	86.5	92.9	E31	71.5	E31	82.1
CT96-2181	E18	E18	75.7	93.6	E17	69.9	E4	75.4
TX96-2184	CA21	CA21	91.6	98.6	CA24	68.2	CA24	75.5
TX97-2320	E18	E18	78.8	92.9	E17	69.7	E17	74.5
NH97-2342	CB3	CB3	77.4	98.5	CB5	67.9	CB1	84.6
PER98-2528	E6	E6	86.0	95.6	CB1	71.6	E29	74.3
PER98-2533	E7	E7	80.4	95.7	E32	68.1	E12	78.6
PER98-2537	E11	E11	78.5	94.3	E19	71.9	E19	82.3
PER98-2558	E33	E33	79.3	96.9	CB1	70.3	E4	75.4

The typing results for the 51 isolates shown in Table 4, fully correlate with the serotype as determined by the conventional neutralization test (Table 4). The nucleotide sequences of the various clinical isolates ranged from 72.4% identity to 95.2% identity with the sequences of the respective prototype strains and only from 63.4% identity to 73.1% identity to the sequences of the second highest scoring

prototypes. The predicted amino acid sequences of the clinical isolates ranged from 88.7% identity to 98.5% identity with that of the cognate prototype strain and from 67.7% identity to 84.6% identity to that of the second highest scoring prototype strain. With one exception, the difference between percent nucleotide sequence identity to the highest scoring prototype and the percent identity to the second highest scoring prototype was 4.2%. In the exception (TX95-2089), typed antigenically as E13, the highest-to-second-highest difference was only 0.9% (72.4% identical to E13 vs. 71.5% identical to EV69), suggesting that either TX95-2089 has diverged significantly from E13 or EV69, or that the E13 prototype strain (Del Carmen) is not representative of the serotype as a whole. When the complete VP1 nucleotide sequence of TX95-2089 was examined, it was found to be 72.6% identical to that of the E13 prototype, 70.1% identical to that of the EV69 prototype (second highest score), and 64.7% identical to that of the E12 prototype (third highest score). The predicted complete VP1 amino acid sequence of TX95-2089 was 88.2% identical to that of E13, 80.8% identical to that of EV69 (second highest score), and 70.0% identical to that of CB1 (third highest score), suggesting that TX95-2089 is probably a strain of E13 which has diverged in nucleotide sequence by accumulating mutations in the third codon position. TX95-2089 was neutralized by monospecific anti-E13 antisera but not by monospecific anti-EV69 antisera (data not shown).

The typing procedure described in this invention contravenes the evaluation of the state of the art in Holland et al. (J. Clin. Microbiol. 36:1588-1594 (1998)), which states that PCR is not able successfully to type enterovirus infections. Furthermore, Oberste et al. (1998a) conducted sequence and phylogenetic analyses of all human enterovirus serotypes based on a portion of the VP2 gene. They determined that this portion of VP2 may be inappropriate for consistent molecular inference of serotype. For these reasons, the method of the present invention, as described above and exemplified in Examples 1-3, provides results that are unexpected by workers in the field.

Example 4. Detection of a Broad Range of Picornaviruses.

The present method has been applied to the detection of a broad range of picornaviruses that afflict both human and nonhuman subjects, according to the procedures generally followed in Example 2.

In addition to the primers 011, 012, and 040, additional primers directed to the detection of human and nonhuman picornaviruses were devised. These are provided as Primer 187 (ACIGCIGYIGARACIGGNCA) (SEQ ID NO:19) that hybridizes to a sequence encoding the amino acid motif TA(A/V)ETGH (SEQ ID NO:83), Primer 188 (ACIGCIGTIGARACIGGNG) (SEQ ID NO:20) that hybridizes to a sequence encoding the amino acid motif TAVETG(A/V) (SEQ ID NO:84), Primer 189 (CARGCIGCIGARACIGGNGC) (SEQ ID NO:21) that hybridizes to a sequence encoding the amino acid motif QAAETGA (SEQ ID NO:85), and Primer 222 (CICCIGGIGGIAYRWACAT) (SEQ ID NO:22) that hybridizes to a sequence encoding a motif M(F/Y)(I/V)PPG(A/G) (SEQ ID NO:86) (see Table 3). Primer 187 is directed to amplification of the CB and E groups in the forward direction (i.e., it hybridizes to the sense strand of the cDNA), Primer 188 is directed to amplification of the poliovirus (PV) group, EV68 and EV70 in the forward direction, Primer 189 is directed to amplification of the group of CA16-like viruses (Oberste et al., J. Virol. 73:1941-1948 (1999)) in the forward direction, and Primer 222 is directed to amplification of all enteroviruses in the reverse direction (i.e., it hybridizes to the antisense strand of the cDNA).

In this example, prototypical serotypes of human enteroviruses were subjected to RT-PCR using, in separate experiments, primer pairs 012/011 (SEQ ID NOs:3 and 4), 040/011 (SEQ ID NOs:3 and 9), 187/222 (SEQ ID NOs:19 and 22), 188/222 (SEQ ID NOs:20 and 22), and 189/222 (SEQ ID NOs:21 and 22). The results are shown in Table 5. Additionally several serotypes from a selection of human and nonhuman picornaviruses, namely bovine enterovirus, human rhinovirus, and simian picornavirus, were examined according to the present method. For simian picornaviruses and HRV2, actual experiments were done. For the other serotypes considered, provision of an amplicon was predicted by comparison of the primer

sequences to each of the viral VP1 sequences. The results of this experiment are shown in Table 6.

Table 5. Amplification of Human Enterovirus Serotypes by Specific Primer Pairs.

Virus	012/011	040/011	187/222	188/222	189/222
CA1	–	–	–	■	□
CA2	□	■	□	□*	■
CA3	–	■	–	□	■
CA4	–	■	–	–	■
CA5	–	■	□	□*	■
CA6	–	■	–	□*	■*
CA7	–	–	±	–	■
CA8	–	□	–	□	■
CA9	■	–	■*	□	–
CA10	–	–	–	□	■
CA11	–	±	–	■	□
CA12	–	■	–	□*	■
CA13	–	–	□*	■	□
CA14	–	■	–	□	■
CA15	–	–	□	■	□
CA16	–	■	–	–	■
CA17	–	±	±	■	□
CA18	–	■	–	(±)	–
CA19	–	±	–	■	□
CA20	–	–	–	■	±
CA21	–	■	–	■	□
CA22	–	–	–	■	□
CA24	–	■	–	■	□
CB1	■	–	■	–	–
CB2	■	–	■	□*	±
CB3	■	±	■*	–	±
CB4	–	–	■*	–	±
CB5	■	–	■	□	□
CB6	■	–	■	□*	□*
PV1	–	■	□	■	□
PV2	–	–	□	■	□*
PV3	–	–	–	■	□
E1	–	–	■	–	–
E2	■	□	■	–	±

Virus	012/011	040/011	187/222	188/222	189/222
E3	■	–	■	–	±
E4	■	–	■*	□	□*
E5	■	–	■	–	±
E6	■	□	■	–	±
E7	■	–	(±)	–	□
E9	■	–	■	–	±
E11	■	–	■*	–	±
E12	■	–	■*	–	□*
E13	■	–	■	–	□
E14	■	□	■	–	□*
E15	–	–	■	–	–
E16	■	–	■	–	±
E17	■	–	■*	–	±
E18	■	□	■	□	□
E19	■	–	■	–	±
E20	■	□	■	□	±
E21	■	–	■	–	–
E24	■	□	■	–	±
E25	■	□	■	–	±
E26	■	–	■	–	±
E27	■	□	■*	–	±
E29	–	–	■	–	–
E30	■	□	■	–	±
E31	■	□	■*	–	±
E32	–	–	■	–	±
E33	■	–	■	–	–
EV68	–	–	□	■	□
EV69	–	–	■	–	–
EV70	–	–	–	■	□
EV71	–	■	–	–	■

CA, coxsackie A virus; CB, coxsackie B virus; PV, poliovirus; E, echovirus; EV, numbered enterovirus. Results are for amplification of prototype strains and/or clinical isolates of the indicated serotypes, based on testing in a standard RT-PCR assay for human enteroviruses (Oberste et al., 1999).

□ and ■ : strong amplification, single band on gel; ■ indicates the primer pair giving optimal amplification for a particular serotype.

± and (±) : weak amplification, single band on gel; (±) indicates the primer pair giving optimal amplification for a particular serotype.

□* and ■* : strong amplification, multiple bands on gel; ■* indicates the primer pair giving optimal amplification for a particular serotype.

– : No amplification observed.

Table 6. Predicted and Observed Results of Amplification of Picornavirus Serotypes by Specific Primer Pairs.

Virus	012/011	040/011	187/222	188/222	189/222
BEV1				[■]	
BEV2a				[■]	
BEV2b				[■]	
HRV1b			[■]		
HRV2			■		
HRV3				[■]	
HRV14				[■]	
HRV16			[■]		
HRV89			[(±)]		
SPV2		■			
SPV9	–	–	–	–	–
SPV10		■			
SPV11	–	–	–	■	–
SPV12	–	–	–	–	■
SPV13		■			
SPV15	–	–	–	■	–
SPV16	–	–	–	–	■
SPV17			■		□

BEV, bovine enteroviruses; HRV, human rhinovirus; SPV, simian picornavirus.

Results are for amplification of prototype strains and/or clinical isolates of the indicated serotypes, based on testing in a standard RT-PCR assay (Oberste et al., 1999) for HRV2, and simian picornaviruses. For the other viruses (indicated by square brackets [ ]), the entry provides a predicted result based on comparison of the primer sequences with the available VP1 nucleotide sequences found in the GenBank database.

□ and ■ : strong amplification, single band on gel; ■ indicates the primer pair giving optimal amplification for a particular serotype.

(±) : weak amplification, single band on gel, optimal amplification for a particular serotype.

– : No amplification observed.

Empty cells indicate primer-template combinations that have not yet been tested.

The results for 012/011 and 040/011 in Table 5 tabulate the observations already discussed with respect to Figure 2 in Example 2.

Taking the results for primer pairs 187/222, 188/222, and 189/222 in Tables 5 and 6 together, it is seen that these primer pairs amplify all human enteroviruses, and five of the six simian picornaviruses tested. They should also amplify the three bovine enteroviruses and all six human rhinoviruses for which VP1 sequences are available in GenBank; other than HRV2, these have not yet been directly tested. Furthermore, the three simian picornaviruses that were not tested using primer pairs 187/222, 188/222, and 189/222 were successfully amplified by primer pair 040/011 (see Table 6).

CLAIMS

We claim:

1. A method for detecting the presence of an enterovirus in a clinical sample comprising the steps of:

(i) obtaining a clinical sample from a subject;

(ii) purifying RNA contained in the sample;

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 gene of the enterovirus genome; and

(vi) detecting whether the amplicon is present;

wherein the presence of the amplicon indicates that an enterovirus is present in the sample.

2. The method as described in claim 1, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

3: The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

4. The method as described in claim 3, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

5. The method as described in claim 4, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

6. The method as described in claim 2, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

7. The method as described in claim 6, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence

comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

8. The method as described in claim 7, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

9. The method as described in claim 1, wherein the amplification procedure comprises a polymerase chain reaction.

10. The method as described in claim 1, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

11. The method as described in claim 1, wherein the detection is carried out by a procedure chosen from the group consisting of gel electrophoresis and visualization of amplicons contained in a resulting gel, capillary electrophoresis and detection of the emerging amplicon, probing for the presence of the amplicon using a labeled probe, and labeling a PCR primer employed in the method and detecting the label.

12. A method for typing an enterovirus in a clinical sample comprising the steps of:

(i) obtaining a clinical sample from a subject,

(ii) purifying RNA contained in the sample,

(iii) reverse transcribing the RNA with primers effective to reverse transcribe enteroviral RNA to provide a cDNA;

(iv) contacting at least a portion of the cDNA with

(a) a composition that promotes amplification of a nucleic acid and

(b) an oligonucleotide mixture wherein the mixture comprises at least one oligonucleotide that hybridizes to a highly conserved sequence of the sense strand of an enterovirus nucleic acid and at least one oligonucleotide that hybridizes to a highly conserved sequence of the antisense strand of an enterovirus nucleic acid, thereby providing an amplification mixture, such that, upon hybridizing, the oligonucleotides direct amplification of at least a portion of the nucleotide sequence of the VP1 gene of the non-polio enterovirus genome;

(v) carrying out an amplification procedure on the amplification mixture, such that, if an enterovirus is present in the sample, an enterovirus sample amplicon is produced whose sequence comprises a nucleotide sequence of at least a portion of the VP1 region of the enterovirus genome;

(vi) determining that the sample amplicon is present;

(vii) determining at least a partial nucleotide sequence of the sample amplicon;

(viii) providing a database consisting of prototypical nucleotide sequences, wherein each prototypical sequence is the sequence of a standard amplicon obtained from a member of a set of prototypical enterovirus serotypes by carrying out the procedure of steps (ii) through (v) on each prototypical enterovirus serotype, wherein each prototypical sequence comprises at least a portion of the sequence of the VP1 gene, and wherein the sequence of each prototypical VP1 gene is different from the sequence of every other prototypical VP1 gene in the database;

(ix) comparing the sequence of the sample amplicon with each prototypical sequence in the database; and

(x) identifying the prototypical sequence that has the highest extent of identity to the sequence of the sample amplicon to provide an identified serotype; wherein the type of the sample is the serotype of the identified serotype.

13. The method as described in claim 12, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

14. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

15. The method as described in claim 14, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

16. The method as described in claim 15, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

17. The method as described in claim 13, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a

motif chosen from the group consisting of the sequences given by SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86.

18. The method as described in claim 17, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

19. The method as described in claim 18, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

20. The method as described in claim 12, wherein the sample is chosen from the group consisting of whole blood or a fraction thereof, a bronchial wash, cerebrospinal fluid, an eye swab, a conjunctival swab, a swab or scraping from a lesion, a nasopharyngeal swab, an oral or buccal swab, pericardial fluid, a rectal swab, serum, sputum, saliva, stool, a stool extract, a throat swab, urine, brain tissue, heart tissue, intestinal tissue, kidney tissue, liver tissue, lung tissue, pancreas tissue, spinal cord tissue, skin tissue, spleen tissue, thymus tissue, cells from a tissue culture, a supernatant from a tissue culture, and tissue from an experimentally infected animal.

21. The method as described in claim 12, wherein the amplification procedure comprises a polymerase chain reaction.

22. The method as described in claim 12, wherein an amplicon encompasses at least a portion of the nucleotide sequence for the VP1 gene of an enterovirus.

23. The method as described in claim 12, wherein the set of prototypical enterovirus serotypes comprises serotypes of coxsackie A viruses, coxsackie B viruses, echoviruses, and numbered enteroviruses.

24. The method as described in claim 23, wherein the serotypes of coxsackie A viruses (CA) comprise CA1 through CA22 and CA24.

25. The method as described in claim 23, wherein the serotypes of coxsackie B viruses (CB) comprise CB1 through CB6.

26. The method as described in claim 23, wherein the serotypes of echoviruses (E) comprise E1 through E7, E9, and E11 through E27, and E29 through E33.

27. The method as described in claim 23, wherein the serotypes of numbered enteroviruses (EV) comprise EV68 through EV71.

28. The method as described in claim 12, wherein determining at least a partial nucleotide sequence of the sample amplicon comprises a sequencing method chosen from the group consisting of a method using 2',3'-dideoxynucleotide chain terminators and a method using chemical degradation of terminally-labeled amplicons.

29. The method as described in claim 12, wherein comparing the sequence of the sample amplicon with each sequence in the database employs a sequence alignment and comparison algorithm.

30. An oligonucleotide comprising, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of a sequence given by SEQ ID NO:80, a sequence given by SEQ ID NO:81, a sequence given by SEQ ID NO:82, a sequence given by SEQ ID NO:83, a sequence given by SEQ ID NO:84, a sequence given by SEQ ID NO:85, and a sequence given by SEQ ID NO:86, or an oligonucleotide complementary to any of them.

31. The oligonucleotide described in claim 30 wherein the oligonucleotide consists of a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, and SEQ ID NO:86, or an oligonucleotide complementary to any of them.

32. An oligonucleotide whose sequence comprises, at the 3' end thereof, a sequence chosen from the group consisting of the sequence given by SEQ ID NO:3, the sequence given by SEQ ID NO:4, the sequence given by SEQ ID NO:9, the sequence given by SEQ ID NO:19, the sequence given by SEQ ID NO:20, the sequence given by SEQ ID NO:21, and the sequence given by SEQ ID NO:22, or an oligonucleotide complementary to any of them.

33. The oligonucleotide described in claim 32 whose sequence consists of a sequence chosen from the group consisting of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:9, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO:22, or an oligonucleotide complementary to any of them.

34. A mixture of oligonucleotides comprising at least two oligonucleotides, wherein at least one of the oligonucleotides hybridizes to a sense strand of a double stranded nucleic acid and at least one of the oligonucleotides hybridizes to an antisense strand of the nucleic acid, the nucleic acid encoding at least a portion of the VP1 gene of an enterovirus, wherein the oligonucleotides hybridize to sequences that are highly conserved among enteroviruses, and wherein the oligonucleotides, when

hybridized to the nucleic acid, direct the synthesis of an amplicon encoding at least a portion of the VP1 protein of enteroviruses when the oligonucleotides are employed in an amplification procedure using the nucleic acid.

35. The mixture of oligonucleotides as described in claim 34, wherein each oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to the nucleic acid.

36. The mixture of oligonucleotides as described in claim 34, wherein the highly conserved sequences occur within the VP1 gene or within about 100 nucleotides from a terminus of the VP1 gene.

37. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif chosen from the group consisting of the sequences given by SEQ ID NO:80 and SEQ ID NO:81, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:82.

38. The mixture of oligonucleotides as described in claim 37, the mixture comprising an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

39. The mixture of oligonucleotides as described in claim 38, wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

40. The mixture of oligonucleotides as described in claim 34, wherein at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif given by SEQ ID NO:86, and at least one oligonucleotide comprises, at the 3' end thereof, a sequence that hybridizes to a sequence encoding a motif whose sequence is chosen from the group consisting of SEQ ID NO:83, SEQ ID NO:84, and SEQ ID NO:85.

41. The mixture of oligonucleotides as described in claim 40, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

42. The mixture of oligonucleotides as described in claim 41, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

43. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:4 and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:9.

44. The kit as described in claim 43, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:3, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:4 and an oligonucleotide whose sequence is given by SEQ ID NO:9.

45. A kit comprising a mixture of oligonucleotides, wherein the oligonucleotide mixture comprises an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:19, an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:20, and an oligonucleotide whose sequence comprises, at the 3' end thereof, the sequence given by SEQ ID NO:21.

46. The kit described in claim 45 wherein the mixture comprises an oligonucleotide whose sequence is given by SEQ ID NO:22, and at least one oligonucleotide chosen from the group consisting of an oligonucleotide whose sequence is given by SEQ ID NO:19, an oligonucleotide whose sequence is given by SEQ ID NO:20, and an oligonucleotide whose sequence is given by SEQ ID NO:21.

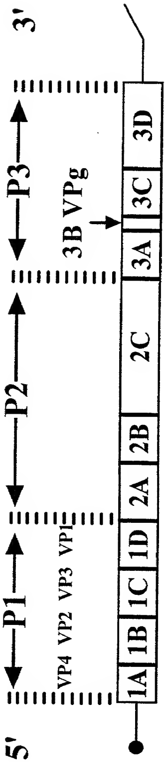

Figure 1

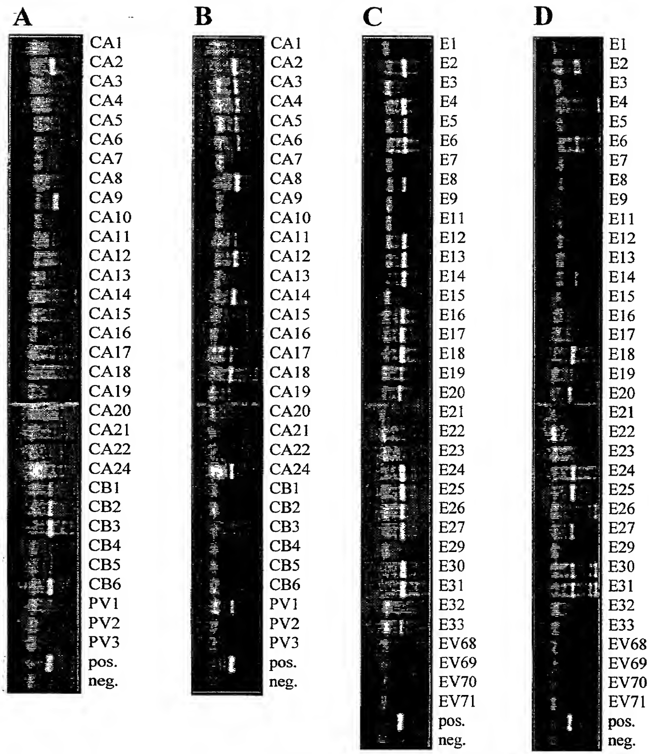
A
CA1
CA2
CA3
CA4
CA5
CA6
CA7
CA8
CA9
CA10
CA11
CA12
CA13
CA14
CA15
CA16
CA17
CA18
CA19
CA20
CA21
CA22
CA24
CB1
CB2
CB3
CB4
CB5
CB6
PV1
PV2
PV3
pos.
neg.
B
CA1
CA2
CA3
CA4
CA5
CA6
CA7
CA8
CA9
CA10
CA11
CA12
CA13
CA14
CA15
CA16
CA17
CA18
CA19
CA20
CA21
CA22
CA24
CB1
CB2
CB3
CB4
CB5
CB6
PV1
PV2
PV3
pos.
neg.
C
E1
E2
E3
E4
E5
E6
E7
E8
E9
E11
E12
E13
E14
E15
E16
E17
E18
E19
E20
E21
E22
E23
E24
E25
E26
E27
E29
E30
E31
E32
E33
EV68
EV69
EV70
EV71
pos.
neg.
D
E1
E2
E3
E4
E5
E6
E7
E8
E9
E11
E12
E13
E14
E15
E16
E17
E18
E19
E20
E21
E22
E23
E24
E25
E26
E27
E29
E30
E31
E32
E33
EV68
EV69
EV70
EV71
pos.
neg.

Figure 2

SEQUENCE LISTING

<110> THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES CENTERS FOR DISEASE CONTROL AND PREVENTION
Oberste, M. Steven
Maher, Kaija
Kilpatrick, David R.
Pallansch, Mark A.

<120> TYPING OF HUMAN ENTEROVIRUSES

<130> 68018/PCT

<140> PCT/USOO/07828
<141> 2000-03-24

<150> US 60/127,464
<151> 1999-03-31

<160> 86

<170> FastSEQ for Windows Version 3.0

<210> 1
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> UNKNOWN

<400> 1
gcrtgcaatg ayttctcwgt 20

<210> 2
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (1)...(0)
<223> Inosine

<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (13)...(0)
<223> Inosine

<223> UNKNOWN

<400> 2
ngcnccdgat tgntgscc 18

<210> 3
<211> 20

<212> DNA
<213> Artificial Sequence

<220> -
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 3
gcnccngayt gntgnccraa 20

<210> 4
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<221> modified_base
<222> (18)...(0)
<223> Inosine

<223> UNKNOWN

<400> 4
atgtaygtnc cnccnggngg 20

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base

<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 5
ggngcrttnc cytcngtcca 20

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (8)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (18)...(0)
<223> Inosine

<223> UNKNOWN

<400> 6
acrtgncnng tytgcatngt 20

<210> 7
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 7
awnttytayg ayggntgg 18

<210> 8
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (5)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<223> UNKNOWN

<400> 8
tananngtnc ccatrttrtt 20

<210> 9
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<221> modified_base
<222> (18)...(0)
<223> Inosine

<223> UNKNOWN

<400> 9
atgtayrtnc cnmcnggngc 20

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 10
ggnggnggrt cngtnakytt 20

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 11
gangaraayc tnatngarac 20

<210> 12
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<223> UNKNOWN

<400> 12
cccatnakrt cnatrtccc 19

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (11)...(0)

<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<223> UNKNOWN

<400> 13
gtrctyacna nnagrtcyct 20

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> UNKNOWN

<400> 14
tsaarytgtg caargacac 19

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> UNKNOWN

<400> 15
stgyccagat ttcagtgt 18

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> UNKNOWN

<400> 16
ggnacncayr tnathtggga 20

<210> 17
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (4)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 17
gccntrttnt grtgnccraa 20

<210> 18
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (12)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 18
ggnacncayr tnrtntggga 20

<210> 19
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 19
acngcngyng aracnggnca 20

<210> 20
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<221> modified_base
<222> (3)...(0)
<223> Inosine

<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 20
acngcngtng aracnggng 19

<210> 21
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (6)...(0)
<223> Inosine

<221> modified_base
<222> (9)...(0)
<223> Inosine

<221> modified_base
<222> (15)...(0)
<223> Inosine

<223> UNKNOWN

<400> 21
cargcngcng aracnggngc 20

<210> 22
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> modified_base
<222> (2)...(0)
<223> Inosine

<221> modified_base
<222> (5)...(0)
<223> Inosine

<221> modified_base
<222> (8)...(0)
<223> Inosine

<221> modified_base

<222> (11)...(0)
<223> Inosine

<223> UNKNOWN

<400> 22
cnccnggngg nayrwacat 19

<210> 23
<211> 888
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA1, strain Tomkins

<400> 23
ggattgggcg attctattga ggctgccatt gacagcatca cacaaaatgc actaaccact 60
gtacaaaata caacacaatc aggacctact cattcaaaag aagttccagc attaacagca 120
gtggaaacag gtgctactag tcaagtagaa ccaggtgact tgattgaaac cagacatgtt 180
ataaacatga gacaaagatc tgaagcatct atcgaatctt tctttggccg atccgcatgt 240
gttgcgatac ttggtttgtc aaacgccaaa ccaactgaca caaacaccaa acaattgttc 300
aaaacatgga gaatatcata tttagaaact caccaactca gaagaaaact tgagttcttt 360
acgtactcaa ggtttgattt ggaaatgacc atagtaatta cagagagggt tttcaatgca 420
gtcaatgtcc cattgcgcaa ttatgtgtac caaataatgt acgttccccc aggtgctcca 480
gaaccacaat catgggatga ttacacgtgg caatcttcta ccaacccatc aatattctac 540
accactggaa atgctcctcc cagagtgtca attccatttg ttggaatagg gtctgcatat 600
tcacactttt atgatggttt ctcacagatt cctcttgact caatcagtgc tggagcaagt 660
aataagtatg gttacacttc aatcaatgac tttggtaccc tggcaattag aatagtaaat 720
gaatatgacc cagtgcaagt ggatgcaaag gcccgagtgt atattaaacc caaacatgtt 780
cgcatgtggt gccccagacc accacgggcc atgccttaca agaatagcac agtggatttc 840
gacccatcag caactgtaat gacccaagtc gcagacatca ggacgtat 888

<210> 24
<211> 882
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA3, strain Olson

<400> 24
ggagatccag tggaagactt aatcgccaat acagttgcta ggactctaga gagaataacc 60
tctccaactc ataatacaac ggcaggcaac accaccgtta gcgagcacag catcggtacc 120
ggttcagtgc ctgcgttgca agctgctgag actggggctt cgtctaacac cacagatgag 180
agtatgatag aaacacggtg tgttgtcaat aggaatggag tgattgagac tagcatcaac 240
catttcttct cccgagcggg gcttgtggga gtgctgaaca tacttgatgg aggcacctca 300
aaaggctttg aagtttggga tatagacatc atgggctttg ttcagcttcg cagaaagcta 360
gagatgttca cctacatgcg gttcaacgct gaattcacct ttgtcgcgac tttgagtgac 420
ggaacaactc cccatataat gttgcaatac atgtatgtgc cccctggagc tcccaaacct 480
caggaaagag attcattcca atggcagact gcaaccaacc catccgtgtt tgcgaaaatg 540
agtgaccctc ctccgcaagt ttcagtacct ttcatgtctc ctgctagcgc ctaccagtgg 600
ttttatgatg ggtacccaac atttgatgat agaccacaga cctctaatcg tccctacgga 660
caatgcccca ataacatgtt gggcacattc gcggtgcgca ttgttagcaa gacgcctgcg 720
gagagagact tgcgcgtccg tgtttacatg aaactgaagc atgtgcgagc atgggtaccg 780
cgacccataa ggtcacagcc ttacgtcttg aagaactacc ccaactatga tggaacccaa 840
atcgtgccca gtgccaaaga tcgagaagac ataaagaaca ca 882

<210> 25

<211> 915
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA4, strain High Point

<400> 25
```
ggtgatgcaa tcgctgatgc tatacaaaac acagttacat ctactataca gagagtcaca      60
accaacactg ttgggcaaga tgcaacagct gctaacacag cacccagctc tcatagtttg     120
aacactggcc tagtccccgc gcttcaagct gctgagacag gagcttcatc cacagccacg     180
gatgggaatt tgattgagac tagatgtgtt gtaaactcca atggtacacg tgaaacccac     240
attgagcatt tcttctctag gtcagggctg gtgggagtta tggaggtaga tgatacgggt     300
actagtggca agggattctc aaactgggac attgacatca tggcgtttgt gcaactgcgc     360
cgtaaactcg aggcatttac atatatgcgg ttcgacgcag agtttacctt tgtcaccaat     420
ttggagaacg ggctcacgaa taatagtgtg atacagtaca tgtatgtacc acctggagcg     480
cctaaacccg atgcccggga atcattccag tggcaaactg caaccaatcc gtcagtcttt     540
caaaaaatgg acagtccgcc acctcaagtt tcagtaccct tcatgtcacc agccagtgcc     600
tatcaatggt tctatgacgg ttaccccacc tttgggcccc actcggagac atctaatcta     660
tcttacgggc aatgtcccaa taatatgctg ggaacattct cggccagggt tgttagcaag     720
caaatcacca atcagaaatt ccagatccgt atttatctac ggctgaagag ggtgagggcg     780
tggatcccca gacctttgag atcgcagccg tacatttaca gaaactaccc cacctatggt     840
actaccatcc aatacctggc caaagatagg cgcaagatca ctgaaactga ttataatgct     900
gaacagcgca cgcat                                                      915
```

<210> 26
<211> 885
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA5, strain Swartz

<400> 26
```
ggcagaccaa ttgcagatat aatagaagga gcagtagctc aaactaccac cagagcacta      60
agtggaccaa ttcagccagt gacagcggcc aacacctctc ccagttcaca tcggcttggt     120
acggggcaag tgccagcttt gcaagcagca gaaacgggag ccacctcgaa tgcgaccgac     180
gagagtttga ttgaaaccag gtgtgtggtc aacagacatg gagtcatgga aactagcatt     240
gaacacttct tttcacgctc aggcttggca ggaattttga taattgagga ctccggtact     300
tccacgaaag gctacgccac ttgggaaatc gatgttatgg gatttgtcca gctgaggcgt     360
aaactagaga tgttcacata catgcgattt gatgcagagt tcacctttat cacagcagaa     420
aggaatggca acaccagccc aatacccatc cagtacatgt atgtcccacc cggagcccca     480
gtccctactg gtagggagac attccaatgg caaacagcga ccaatccatc cgtgatctca     540
aagatgactg atccaccagc ccaggtgtct gtaccattta tgagcccagc cagtacttat     600
caatggttct acgatggcta ccccacgttc ggagaagttc cagtgactac gaacttgaac     660
tatggacagt gcccaaacaa caaaatgggc actttctgca tccgcatggt ctcaggtgta     720
tctacaggca aggacgtcac tgtgcgcatt ttcatgaagt tgaagcatgt gcgcgcctgg     780
gtgccaaggc ccatcaggag ccagccttac ttgttaaaga attatcccaa ctttgacaag     840
tcaaatattg tagacgcatc atcgaacagg acatatacca ccact                     885
```

<210> 27
<211> 915
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA6, strain Gdula

<400> 27
aatgacccca tttcaaatgc aatagaaaat gctgtgagca cactcgctga caccacgata 60
tcacgtgtta cagcggccaa cactgctgct agctcccatt cccttggtac tggacgcgtg 120
ccggcgttgc aggctgcgga gacaggggca agttccaacg ctagcgatga gaacctgatt 180
gaaactcgtt gtgtgatgaa tagaaatgga gttaacgaag caagtgtaga acacttctac 240
tcccgtgcag ggctagtagg agttgtggag gtgaaagact caggcactag tcaggacggg 300
tacacggtgt ggcccataga tgtgatgggc tttgtgcaac agcggcgcaa gttagagcta 360
tctacttaca tgcgctttga cgctgaattt acctttgtgt ccaatctcaa tgacagcaca 420
acacccggca tgctattgca gtacatgtac gtgccgccgg gtgcgcccaa accagacggt 480
aggaagtcat atcaatggca aacagccacc aacccttcaa tattcgcaaa gttgagtgac 540
ccaccgcccc aagtgtctgt cccattcatg tcaccggcgt cagcctacca gtggttctac 600
gatggttacc ccacgtttgg cgaacacaag caagctacta atttacaata cggtcagtgc 660
cctaacaaca tgatggggca ttttgctatt cggacagtta gtgaatccac caccgggaaa 720
aatgtccatg tccgggtgta catgagaatt aagcacgtaa gagcatgggt gcccagacct 780
ttcagatccc aagcttacat ggtcaaaaac tacccgacat acagccaaac aatatccaat 840
actgcagccg atcgtgcgag cataaccact acggactatg agggtggcgt accagcaaac 900
ccgcagagaa ctttt 915

<210> 28
<211> 888
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA7, strain AB-IV

<400> 28
ggagacgaaa tactcgacct aatcgagagt gctgtacaga ataccactaa agccattacc 60
agctcaatcg acaccaaaac tggtgctaac actcaagcta gccaacatcg tataggcttg 120
ggggaggttc ccgctcttca agctgctgag acaggatcgt cttcgctcgt ttcggacaag 180
aacatgatag aaacaaggtg tgtcgtaaac aaacacagca cagaggaaac cagcattaca 240
aacttctact ccagggcggg cctagtgggg gttgtgaaca tgccagtaca aggaaccagc 300
aacacaaagg gtttcgcaaa gtgggggata gatataatgg gctttgtgca gatgaggcgc 360
aaacttgagc tcatgacata catgagattc tccgccgagt ttacgttcgt acccagcact 420
cctgggggag agactactaa ccttatactg caatacatgt atgcacctcc cggagctccg 480
ctgccaacca ggcgggattc atacgaatgg caaacatcca ctaacccctc tattatcagc 540
aagatggcgg acccaccccgc tcaggtatcg gttccattcc tttctcctgc atcagcatat 600
cagtggttct atgatggcta ccccacattt gggaaacacc caatagatca ggacttccaa 660
tatggcatgt gcccaaacaa catgatgggc acattctgtg tgcgcatgat cggtgggggc 720
aaaccgaccc aatcagttac catacgtata tacatgagat taaagcatat ccgtgcatgg 780
gtgccccggc cactgaggag tcagaattac actatgagga attacccgaa ctacaacggg 840
ggcgcaataa aatgtacatc aaaaagcaga gctaccataa caacctta 888

<210> 29
<211> 882
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA8, strain Donovan

<400> 29
ggagattcca ttgaagacat aataagcaac actgtcaccc gtacactgca acaaatcagt 60
gccccatcac acgacactac agcagccaac acctcagtga gtaatcataa aattggtacg 120
ggggatgtcc cagctctcca agctgcagag actggcgcta cttccaatgc ctcagacgag 180
aacatgattg agacacgatg tgtgttaaat cgcaatgggg ttgtggaaac tagtttggac 240
catttctttt caagagcagg ccttgtggga gtgatcaatg tgcaagatgg cggcactcag 300
aagggttttg aagtgtggga catagatgtc atggggtttg ttcaactcag gaggaagttg 360
gagatgttca cgtacatgag gttcaacgcc gagttcacat tcgtatccac actcgcggat 420

```
ggcacaactc ccagagtgat gttgcagtac atgtacgttc cacctggtgc ccccaaacct    480
caggagagag attcgtttca gtggcaaact gcaaccaacc catcagtatt ttgcaaaatg    540
agtgaccctc ctccacaggt ttccgttcct ttcatgtcac cagctagtgc ctaccaatgg    600
ttctacgatg ggtacccaac attcgatgat cgaccggcca cctcaaacca cccgtacggt    660
cagtgcccca ataacatgat gggcacattc gcagtgcggt ttgtcagcaa gaccccagcc    720
acacgggatc tgcgtgtcag agtgtacatg cgcctgaaac acgtgcgcgc atgggtaccg    780
agacctatcc gatctcaacc ctatattttg aaaaactacc caaattatga tggcacaaag    840
ataacgtcga catctaagga taggcaaagc atcaaaacaa ca                       882
```

<210> 30
<211> 894
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA10, strain Kowalik

<400> 30

```
ggcgaccccg tggaggacat catccacgac gctttgagca gcactgtgcg gcgggccata     60
actagtggtc aagatgtcaa cacagcggcc ggtaccgctc ctagctctca caggttggag    120
actggtcgtg ttcccgccct acaagcagca gaaactggag ccacttctaa cgctacagat    180
gagaacatga tagaaacgcg gtgtgtcatg aacagaaatg gagtgttgga ggcgactata    240
agtcatttct tctcacgctc aggtttggtg ggtgttgtca atctaactga cggaggcacc    300
gatacaacgg gatatgcagt gtgggacatt gacatcatgg gttttgtgca actgcggcgg    360
aaatgtgaga tgttcacata catgagattc aacgctgagt tcacattcgt cactacaaca    420
gaaaatggcg aggcaaggcc atttatgtta cagtatatgt atgtacctcc aggtgcccct    480
aagccaacgg gtagagatgc ttttcagtgg caaacagcga caaatccatc cgttttcgtt    540
aagctcacag atccacctgc tcaggtatca gtccccttca tgtcacctgc tagtgcctac    600
caatggttct atgacgggta tccaacattt ggacaacacc cggaaacatc taatacaaca    660
tatggacagt gccctaacaa catgatgggg acctttgctg tgagagtagt gagtagagtg    720
gctagccagc tcaaactaca gacacgagtg tatatgaagc ttaagcatgt gagagcatgg    780
atccctaggc caataagatc ccagccttac ctcctaaaga attttccaaa ttatgatagt    840
agtaagatca catacagcgc aagagatcgt gccagcataa aacaagctaa tatg          894
```

<210> 31
<211> 912
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA11, strain Belgium-1

<400> 31

```
gggccaatag aagaaatcat ctcaactgtt gccagtaacg cgttggcgct cagtcaaccc     60
aagccagtgg acaactctgt acaaaacacc caacaaagtg ctccagtgca tagccaggag    120
gtgccagcat tgaccgcagt ggagacaggg gcgacaagtg atgtggttcc atctgaccta    180
attcagacta gacacgtatt gaatgttaaa tccaggtctg aatccaccat cgagtcattt    240
tttgcaagag ctgcatgtgt aaccattatg caggtggaca atttcaacgc aacctctgtg    300
gaagacaaaa gaaagttgtt tgctaaatgg gcaatcacct acactgatac cgtccagctg    360
agacggaaat tagagttttt cacttattct agatttgact tagagatgac ttttgtgcta    420
actgagagat actactccca aagctcaggg catgctagat ctcaggtgta ccaaattatg    480
tatgttccac caggggcacc cacgcctagt gcatgggacg actacacatg gcaaacatcc    540
tccaacccat ccatttcctt taccaccggc aatgcaccac cgcgcatttc aattccattt    600
gttggaatcg ccaatgcata ctcacacttt tatgatggct ttagtagagt acctttggag    660
ggagaaacaa cagacacagg agacgcttac tacgggctca cttcaataaa cgattttggt    720
acacttgcag tcagggtagt taatgactac aacccagcca gggtggagac aaggattaga    780
gtatacatga agcccaaaca tgtgagagtc tggtgcccgc gacctccaag agcggtaagc    840
tacagaggac ctggagtcga cctcctatca acatcagtaa cacctttatc caaacatgac    900
```

```
ctagcgacat ac                                                          912

      <210> 32
      <211> 888
      <212> DNA
      <213> Enterovirus

      <220>
      <221> misc_feature
      <222> (0)...(0)
      <223> CA12, strain Texas-12

      <400> 32
ggagatacag tgagtgatat gatcgaaaat tccatcaacc gaattaccag tgcaatttcc      60
actacccaga cacaccagac agcagctgac actagagtta gtacacacag gttaggcacg     120
ggggaggtgc cacctttaca agcagcagag acaggtgcca cctccaacgc aaccgacgag     180
aacatgattg aaacacgctg tgtcgtcaac aggcacgggg tgagcgagac cagcgtggaa     240
tacttcttct ctcgctctgg tttggcagga atagtcatcg tggaggatgc aactgccact     300
aataagggtt atgccacatg ggagattgat gtcatggggt tcgcgcaact gcgtcgcaag     360
ctggagatct tcacatacat gcgcttcgat gcagagttca cttttgtggc aacagaacgc     420
aatgggagca ccagcccggt catgatgcag tacatgttcg tgccccctgg cgcccctgtt     480
ccaacaggga gagatacctt ccaatggcaa tctgctacta acccttcagt gctagtaaaa     540
atgacggatc caccggccca agttgccatc ccctttatgt ctccagctag tgcataccaa     600
tggttctatg atggatatcc tacctttgga gaaagaccag ttacaaccaa catgaattat     660
ggacagtgtc ccaacaacaa aatgggaact ttttgtatac gcactgtctc cggtgaagcg     720
tcagggaaaa acatcactat acgtattttt atgaggttga agcatgtaag agcgtgggtg     780
cctcgcccaa ttagaagcca gctatatctg cttaaaaatt accccaactt tgataacact     840
aagatcctca acgcctccca caacagagct tctatcacat caaacaca                  888

      <210> 33
      <211> 927
      <212> DNA
      <213> Enterovirus

      <220>
      <221> misc_feature
      <222> (0)...(0)
      <223> CA13, strain Flores

      <400> 33
gggttggaag atctaataca acaagttgcg tctaacgcat tacaattgtc ccagccaaca      60
agaccggcac tcccaccagc cgagcagagt gtccccaaca ctaaccaaac aactccagaa     120
cactccaagg aagtcccagc gttaacggca gttgaaactg gcgccacgaa tcctctagag     180
cctggcgaca cagttcagac tagacatgtg atacaaacta gaagtagaag tgaaagtaca     240
gtggagtctt tctttgcgcg aggtgcatgt gtaaccatta tgggagtgga caactataat     300
gagacattga aaggagacca gaagtctact ctatttacaa cctggaacat cacctacact     360
gacacagtcc agctacggag aaaactggaa atgttcactt actccaggtt tgacatcgag     420
tttacttttg tggtgactga acgctactac tcatcaaaca gtgggcatgc tctgaaccaa     480
gtgtaccaaa ttatgtatgt accacctgga gcaccagtgc caaagaaatg ggatgattac     540
acctggcaaa cctcttcaaa cccgtccata ttctacactt atgggtcagc accacccagg     600
atatccatac cctttgtggg tatagcaaac gcttactccc acttctatga tgggtatgcg     660
acagtgccct tgaaaactga caccacagac tcaggagcag cctactatgg agcagtatcc     720
ataaacgact tcggactgct tgcagttcgc gtcgtcaatg aacataatcc agtcagagta     780
tcatccaaaa ttagagtgta tatgaaacca aaacatgtca gggtatggtg tcccagacct     840
ccaagggctg tagagtatta tggaccagga gtggactaca aggcaaacac tttaacaccg     900
ttgccaataa agaatttgac tacttat                                         927

      <210> 34
      <211> 888
      <212> DNA
      <213> Enterovirus

      <220>
```

<221> misc_feature
<222> (0)...(0)
<223> CA14, strain G-14

<400> 34
ggtgacaaag tggcagacat gattgagacc gcagtggaga agaccgtgtc ctcactaact 60
tcccctattc aaacccccac agccgccaac acaaacgtga gtaatcatcg aattgagctg 120
ggggaagtcc cggctttgca agctgctgaa accggcgcga cgtctcttgt gtctgatgaa 180
tacttgatag agactcgttg tgtagtgaat agccatagta cagaggaaac tacagtgggg 240
cacttctttt caagagcggg gttggtggga gtgattgacc tcccattaca gggaacagtc 300
aacacaggag gattcgcctc gtgggatatt gatgtaatgg gatatgttca gatgagaagg 360
aaacttgagc tgttcacata tgcccgcttc gatgcggagt ttaccttcat agcttccacc 420
ccagatggcg aggtgaagcc agtgttctta cagtacatgt tcgtcccccc tggtgcacca 480
aaaccaacag ggcgcaacac ctacgaatgg caaactgcaa caaacccttc tgtgttggtc 540
aagagcacag atcctccagc acaagtctct gtaccgttca tgtcaccagc cagcgcatat 600
cagtggttct atgacgggta cccaaccttt ggaaagcacc tgcctgctga tgactttcag 660
tacggtatga ccccaaataa catgatggga tcgttctgtg ccaggatagt gggggaagga 720
gcgcctagtg tacacttggt tatccgtatc tacatgcgca tgaaacacgt gcgggtgtgg 780
attccacgac ctatgcgcag ccagccatac gttgcgaaga attaccctaa ctacaagggt 840
tctgagatca agtgcgcatc atctagtcgt aagtcaatca ccacatta 888

<210> 35
<211> 912
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA15, strain G-9

<400> 35
gggccaatag aggagatcat ctcgaccgtc gccagcaatg cacttgccct cagtcagcct 60
aaaccggtgg ataattctgt acaaaacacc caacagagcg cgcccgtgca cagccaagag 120
gttccagcat taacagcagt agagactgga gcaacaagtg atgtggtgcc agctgatcta 180
gtgcaaacca ggcatgtagt gaatgtcaag tccagatctg agtccactat cgagtcgttc 240
tttgcaagag ctgcctgcgt gactattatg caggttgata actttaatgc caccaccacg 300
gaggacaaga ggaagttatt tgccaaatgg gccatcacat acacagacac agtacaattg 360
aggaggaaat tggaattttt cacgtactcc aggttcgatc ttgagatgac tttcgtgcta 420
actgaaagat actattctca gagctcggga cacgctagat cgcaggtgta tcaaatcatg 480
tacgtccctc caggagcacc aacaccaaat gcatgggatg attacacgtg gcagacgtct 540
tctaacccat caattttctt caccactggt aacgcacccc cacgggtttc aatcccattt 600
gtgggcattg caaatgctta ctcacacttt tatgatggct tcagcagggt acctttggaa 660
ggagagacca ctgactcagg tgacgcttat tatggcctca cttctatcaa tgactttgga 720
acacttgcag taagagtggt caatgactac aacccagcga gagtggagac aaggatcaga 780
gtctacatga aacctaagca tgtgagagtg tggtgtccac gaccccctag ggctgtgagc 840
tacagaggac ccggtgtgga cctactgtcc acctcagtga cgcccctatc taagcatgaa 900
ttgacaacgt ac 912

<210> 36
<211> 918
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA17, strain G-12

<400> 36
ggcattgaag acttgatcca acaggttgca tcgaatgcgc tgcaaatctc acagccgacg 60
cgtccggcac tgccctctac agaaagtctt cccaacacac aacaatcggc accttcgcat 120
tctcaagagg tcccggcgct gacagcagtt gagacaggcg cgacaaatcc attggagccg 180

```
tctgacacgg tacaaacaag gcatgttatc cagactagat ccaggtcaga gtccacaata      240
gagtccttct tcgcgcgtgg tgcatgtgtg acaatcatga cagtggaaaa ttttaacgcg      300
actgaggcgg cagacaagaa aaagttgttc gccacttgga atattacata cacagacaca      360
gtgcagctca gaaggaagtt ggagatgttc acttactctc gatttgacat tgaatttacc      420
tttgtcacca cagaaaggta ctacgccagt aactcaggcc atgcgcgtaa tcaggtttac      480
caactcatgt atgtaccccc aggagcccct gtgccacaac aatgggatga ttacacgtgg      540
caaacttcct ccaacccatc ggtgttttac acatacggtg acgctccagc gcgcatttcc      600
ataccatttg tagggatagc taatgcctat tcccactttt atgacggcta tgcagtggtg      660
ccattgaaag attccaccca ggatgctggt gctgcctatt atggtgcaac ctcaattaat      720
gattttggaa tgttggcggt gagagtagtc aacgaattca acccagccag aatcacatct      780
aaattgagag tgtacatgaa accaaagcat gttagggtgt ggtgtcctag accaccaagg      840
gtggtgccgt acttcggacc cggtgttgat tataaggata gtttgacacc gctttctaca      900
aaagcactca acacttat                                                    918
```

<210> 37
<211> 927
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA18, strain G-13

<400> 37

```
ggcttggaag acctcatcca acaagtggcc acgaatgcat tgagtctgtc gcagcccaca       60
agacccgcac ttccaccagc agaacaaagt gtgccaaaca ccagtcagac caccccagaa      120
cattcaaagg aagtacccgc actcactgca gtggagaccg gtgcaaccaa cccattggaa      180
ccaggtgaca cagtgcaaac tagacatgtt gttcaaacaa gatcaaggag cgaaagtacg      240
gtggaatctt tctttgcaag aggggcgtgt gtcacgatta tgggagttga caattacaat      300
gaaagcttga ccagtagtca aaaatccacc ctattcgcca cttggaatat tacatacact      360
gatacagtac agttgaggag aaaattggaa atgttcacct actccagatt tgacattgaa      420
tttaccttcg tagtaactga acgttactac tcgtcaaaca gtggccatgc cttgaatcag      480
gtgtatcaaa tcatgtatgt gccaccaggc gctccaattc ctaagaagtg ggatgattat      540
acctggcaaa catcatcaaa cccctcaata ttctacacct atggaacagc accacccaga      600
atttcgatcc cttttgtggg cattacaaac gcgtactcac atttttatga cggatatgcg      660
actgtaccac tcaagacaga cactacggat ccgggggcgg ccttctatgg agcagtttcc      720
atcaatgact ttggtttgtt ggcggtgcga gttgtcaacg agcacaaccc ggtaagagtg      780
tcttcaaaga taagagtgta catgaagcct aaacatgtca gagtgtggtg cccacgacca      840
ccacgtgccg tggagtacta cggaccaggg gtagattaca aggcaaacac attgacacct      900
ctccctacca agaacttaac tacttat                                          927
```

<210> 38
<211> 888
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA19, strain 8663

<400> 38

```
ggtattgatg atatcataga taatgttgta accaatgctt tgaaggtgtc catgccacaa       60
gttcaagata cgcaatctag tggaccagtt aactcaaaag aagtacctgc attaacagct      120
gttgaaacag gggctactag tcaagttgac ccatcagacc taatagaaac tagacatgtt      180
attaataacc gcctcagatc tgagtgcaca atagaatcat tctttgggag gtcagcatgt      240
gtggccataa ttgggttatc taaccaaaaa cccaccagtg acaatgcagc caagctcttt      300
gctacatgga agattagtta tcttgatatg tatcaattga gaagaaaatt ggaattcttc      360
acatactcca gatttgatct tgagttaacc tttgtaattt cagaaagatt cttcacctca      420
acttcagctg ctgcaagaga ttatgtatac cagatcatgt acattccccc aggagcccct      480
atccctcagg tatgggatga ttacacatgg caatcatcca caaacccctc aatattctac      540
accacaggaa atgcatgccc tagagtgtcc atccctttg ttgggatcgg tgcagcatac      600
```

tctcacttct atgatggatt ctctttagta cctttcaata ccatcgatgc tggtgcttca 660
aacaggtacg ggtacaccac cataaatgat tttgggacta tggcaatcag gatagttaat 720
gaatacgacc cagtcacaat tgatgcaaaa gtcagggttt acatgaaacc aaagcatatt 780
aaggtgtggt gccccagacc tccacgggca gtagcataca atgggccaac agtgaatttt 840
aatgaaaacc cccatgtaat gacagcagtt gctgatatta gaacttat 888

<210> 39
<211> 909
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA20, strain IH-35

<400> 39
ggtatcgaag atcttatcac cgaagttgca agcaacgctc tgaagttgtc acaaccaaaa 60
cccagcacac aacagagttt accaaacact agtagctcag aaccaactca ctctcaggaa 120
gcgccggcat tgaccgcagt agaaacagga gcaactagta gcgtagtacc agctgatctg 180
gtccagacgc ggcatgtgat acaaacacgt agccgaagtg agtctacagt tgagtcattc 240
tttgctcggg ggcgtgtgt aacaatcatg tcagtggaaa attacaatga aaccgctatc 300
gcagagtcca aattatttac caagtggaac attacctaca cagacacagt ccagttgaga 360
agaaaactag agatgttcac atactccaga tttgatattg agttcacatt tgtggtgact 420
gagcgttacc actccgcaaa ctcaggtcat gcactaaatc aagtttacca gatcatgtat 480
gttcctccag gtgcaccagt gccacaaaga tgggacgact acacatggca aacgtcatcc 540
aacccctcag tcttttatac ctatggtaca gcaccagcca gaatatcgat tccatatgta 600
ggcatagcca atgcctactc gcatttttat gatggcttcg ccaaagtgcc cattgaaggc 660
gagacgtcag atccaggtga tgcatactat ggtgcaacgt ccatcaatga tttcggcatc 720
ttagccatac gtgtggtcaa cgaacacaat ccagtgcaag tttcttccaa gattagagtg 780
tacatgaaac ctaaacatgt gcgcgtttgg tgtcccagac cacctagagc tgttccatac 840
tttggccccg ggttgatta taaaggtgac gccctcacac cactatcacg caaggattta 900
accacctat 909

<210> 40
<211> 888
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA22, strain Chulman

<400> 40
gggattgagg atacaatcga aaaagtggtt ggtgatgctc taagggtctc aatgccacaa 60
gttgccaaca cccagccatc aggacccgta aattctaagg aagttccagc actgacagca 120
gtggaaacag gtgcaaccag tcaagtcacc cctgaagatt tgatcgaaac caggcatgtt 180
attaacaata gactaag[illegible]tc tgagtgcact gtggaggcct tctttggaag gtctgcatgt 240
gttgccatcc ttggt[illegible]gt aaacaaaaag ccagacacca caaatgccaa agacctcttt 300
acaacatgga gga[illegible]cta cctgcaaact tatcaactga ggaggaaact cgaactcttc 360
acgtattcta gatt[illegible]tt ggaattaacg tttgtcatta cagaaagata cttttcaggg 420
acagcagcca caacca[illegible]ga ttatgtttac caaataatgt atgtaccacc aggagccccc 480
ataccaaata cctgggacga ctacacctgg cagtcatcta ccaacccctc tgtcttctac 540
accacaggca atgccagccc acgcatgtct ataccctttg ttggtattgg tgccgcctat 600
gctcactttt atgacgggtt cagtgtggta ccattcaatc aaatagatgc aggagcatcc 660
aacaaatatg gctactcatc aatcaaagac tttggtacat tggcagttag aattgttaat 720
gagtttgatc cagtgacaat agaggctaaa gtcagagtgt acatgaaacc caaacatgtc 780
agggtgtggt gtccaagacc acctcgtgca gtaccatatc aaaactcatc agttgatttc 840
gcccaaaacg cagtagcaat gaaccaagta gccacaatta ggacgtat 888

<210> 41
<211> 915

<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CA24, strain Joseph

<400> 41
ggtatcgaag ataccattga cactgtcatt aacaatgccc tacaactatc tcaaccacag 60
ccaaataagc agttgacagc tcagtctacc ccctccacaa gtggagtaaa ctcccaggag 120
gttccagctc tgaccgctgt ggaaaccggt gcctcgggac aagcagtgcc cagtgatgtg 180
attgagacca gacacgtggt taattataag acccgatctg aatctactct tgagtctttc 240
tttggaaggt cagcttgtgt caccataatt gaggtcgaga acttcaatgc cactagtgaa 300
gcagacaaga ggaaacagtt caccacttgg ccaatcacat acaccaatac cgtgcaattg 360
cgcaggaaac tagaattctt cacttactcc aggtttgacc tagagatgac ctttgtagtg 420
acagaaagat attatgccag caacacaggt cacgccagaa accaagtgta tcaaataatg 480
tacattcctc ctggtgcacc acaacccaca gcatgggatg attacacgtg gcaaagctct 540
tcgaatccgt cagtctttta cacttatggg agtgctccac ccaggatgtc tataccgtat 600
gtcggtatcg caaatgcata ctctcttttt tatgatgggt ttgcacgagt accactgaag 660
gacgaaacag cggactcagg tgatactttt tacgggctag tcaccatcaa tgattttgga 720
accttagcaa taagagtagt gaatgaattt aacccagcta ggattacatc aaaaattaga 780
gtgtatatga aaccaaagca tgtaagatgc tggtgcccta gaccaccacg tgcagtgcca 840
taccgtggtg aaggagtaga ttttaattca agttcaatca caccactaac agcagtcgca 900
aacatcaaca cattc 915

<210> 42
<211> 852
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CB2, strain Ohio-1

<400> 42
agcccagtgg aggaatccat tgagagaagc attggcagag ttgctgacac cattggtagt 60
ggaccatcca attcggaggc aataccggca ctcacagcag tagaaacagg acacacatca 120
caggttacac ctagtgacac gatgcaaaca agacatgtgc acaactacca ttcaaggtcc 180
gaatccagcg tagagaactt cctggcacgc tcggcttgtg tgttttatac aacatacacc 240
aacggtaaaa aaaaaaatgc cgccaaagag aagaagtttg caacgtggaa agtgagtgtt 300
agacaagccg cccaactaag aagaaagcta gagttattca catacttacg ctgtgacatc 360
gaattaacat tcgtcatcac cagtgcacaa gatccatcga ccgctaccaa cttggatgtg 420
ccagtgttga cccatcaaat aatgtacgtc ccacctggtg gtccagtccc tgaaaccgtg 480
gacgattaca actggcaaac atctacaaat cccagccttt tttggactga agggaatgca 540
cctccacgca tgtcaattcc attcatgagc ataggcaatg cctatagtat gttctatgat 600
ggttggtccg agtttaggca tgacggtgtg tacggcctga atacccttaa caatatgggc 660
acaatatatg ctaggcacgt caacgctgac aacccaggta gcatcaccag cacagtgaga 720
atatacttca aacccaaaca tgtcaaggca tggattcctc gcccgcctcg tttggcacag 780
tatcttaaag ccaataatgt gaattttgag atcaccgatg tgacagaaaa gagagatagt 840
ctcacgacca cg 852

<210> 43
<211> 846
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> CB6, strain Schmitt

<400> 43
agcccagtgg agggcgccat agagagagcc attgcacggg tcgctgacac tatgccaagt 60
ggcccaacca attcagaagc agtgcctgcc ctgacagcag tggaaacggg ccacacctcc 120
caagtcgtcc ccagtgataa catgcaaacc aggcacgtga agaagtacca ttcacgctcc 180
gaaaccagcg tcgagaactt tctgtgtagg tctgcatgtg tatattttac cacatataag 240
aaccagacag gggcgaaaaa tagatttgct tcttgggtaa tcaccacaag acaagtggcc 300
cagctcagga gaaaactaga aatgtttacg tacttgcgtt tcgacattga actcaccttt 360
gtcattacaa gtgcgcaaga ccaatccact atttcccaag acgcccctgt gcagacacat 420
cagataatgt acgtgccacc gggaggccca gtgccaacca aagttgacga gtatgtgtgg 480
caaacatcca ccaaccccag cgtcttttgg accgagggta acgctccacc acgtatgtca 540
gttcccttta tgagtatcgg taatgcttat agcacatttt atgacgggtg gtctgatttt 600
tcaaacaaag gaatatatgg gttgaacacc ttgaacaaca tgggaacatt gtacatccgc 660
cacgttaacg ggcccaaccc agtaccaatt accagcacag tgaggatata ctttaagccc 720
aagcatgtta aggcctgggt gcctaggcct ccaaggcttt gccagtacaa aacgtttagg 780
caagtcaact ttacagtgac tggagtgacc gagagtaggg caaatataac caccatgaat 840
actaca 846

<210> 44
<211> 852
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E1, strain Farouk

<400> 44
ggtgatgtgc agaatgctgt cgaaggggct atggtcaggg tggcagatac agtgcaaact 60
tcagccacaa actcagagag ggtgcctaac ttgacagcag tagaaactgg tcacacttcg 120
caggtagtac ctggtgatac catgcagact agacatgtga tcaacaatca cgtgaggtca 180
gaatctacaa ttgagaactt ccttgccaga tcagcgtgtg ttttcttcct agagtacaag 240
acagggacca aagaggattc caatagcttc aacaattggg tgattacaac caggcgagtg 300
gctcaactac gtagaaaact ggaaatgttt acttacctac ggtttgacat ggaaatcacc 360
gtggtcatta caagctcgca agatcagtct acatcacaaa accagaatgc accagtgcta 420
acacaccaga taatgtatgt accaccaggg ggacccatac ccataagcgt ggatgattac 480
agctggcaaa catccaccaa ccccagtatc ttttggaccg aagggaacgc tccggcacgc 540
atgtcaattc catttattag cataggcaat gcgtatagta atttctacga tgggtggtct 600
cacttctccc agactggcgt gtatggcttc actactctga acaacatggg tcaattgttc 660
ttccggcacg taaacaagcc caacccagcc gctattacaa gtgtggcgcg catttacttc 720
aaaccgaaac atgtacgcgc ttgggtgcct agaccaccgc gcttgtgtcc atacatcaat 780
agcacgaatg tcaactttga acccaagcca gtgactgaag tacgtaccaa cataataaca 840
acgggtgcct tc 852

<210> 45
<211> 882
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E2, strain Cornelis

<400> 45
ggagatgagg tgaagcatga acccacagtg gccaacacaa cagcaagtgg accatcaaat 60
tcacaacaag taccggcact cacagcagtg gagactgggc acacctcaca ggtggttcca 120
agcgatacca tacaaaccag acatgttcac aattaccata gtagaactga atccaccctg 180
gagaacttcc tcggaagatc agcatgcgtg cacattgact cgtataagac caagggagtg 240
accggcgaga gcacccggta cgcatcatgg gagatcacca ctcgcgagat ggtgcagctg 300
cggaggaagt gtgaactctt cacctacatg cgatatgatc tagaaatcac gtttgtgatt 360
acaagtcgcc aggagcaagg ggccaaactg tcgcagaaca tgccagtatt aacacatcag 420
atcatgtatg tcccaccggg cgggcctata ccaaccagca acgagagtta cgcttggcaa 480

acgtcaacga acccaagcgt gttttggaca gaaggaagct cgccaccacg aatgtcaata 540
ccgtttgtta gcataggaaa cgcatacagc aatttctatg atgggtggtc gcacttctca 600
caaaacggtg cgtatggtta cacggcacta aacaagatgg gtaggatatt cgtgcgccat 660
gtaaacaaag agacaccact gcaagtcata agcacaatac ggatgtatat gaagcccaaa 720
cacgtgcggg cttgggtgcc aagaccacca cgcctgtgtc catacctgcg ggcgggtgat 780
ataaactttg aagtgactga tgttacagaa aaacgaaata acatcaatta tgtcccaacc 840
ccatcccaca gcagcagtgt gcacatgcgc ttgaacaacc at 882

<210> 46
<211> 879
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E3, strain Morrisey

<400> 46
ggggacgtcg aagaggcaat tgatagggca gttgcgaggg tggctgacac aatgccaacc 60
ggtccacgaa acactgagag cgtgcctgcc ctgacagcag tagagacagg ccacacctca 120
caggtcgttc ctggtgacac aatgcagacg aggcatgtta agaactatca ctccaggaca 180
gagtcatcaa ttgaaaactt cctgtgcagg gctgcgtgcg tgtatataac aacatacaaa 240
tcagctggtg gaacacccac agagcgatat gcaagttgga ggataaacac caggcaaatg 300
gtgcagctca ggaggaaatt tgagctcttc acatacttgc gctttgacat ggaaatcaca 360
tttgtgatca caagcacaca agatcctggg acacaattgg cacaagatat gcctgtacta 420
actcatcagc tcatgtatat cccacctggg ggccctgttc ctaacagtgc cacagatttt 480
gcatggcaat catcaactaa tccaagtata ttttggacgg aaggctgtgc tccagcacga 540
atgtcggtgc cgttcatcag cattggcaat gcctacacca atttttacga tgggtggtcg 600
catttcaccc aagaaggggt ttatgggttt aactcactga acaacatggg ccacatatat 660
gtgaggcacg tcaatgagca aagcctgggt gtctcgacca gcaccgttcg cgtgtatttt 720
aaacccaaac atgtgcgtgc ttgggtacca agaccaccca gactgtgccc atacactaag 780
agttcaaatg tgaatttcaa accgaccgct gtcactgatg agcgaaagga tatcaacgat 840
gtaggcaccc ttcgaccaac agtgtacact aaccttgtg 879

<210> 47
<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E4, strain Pesacek

<400> 47
ggagacgtgc aagatgcagt gacaggtgct atagtacgtg tcgctgacac tctcccaaca 60
ggtccctcaa ataatgaagc tatacccaat ttaacagcag tggagactgg ccatacctcg 120
caagtgacac caggcgacac aatgcaaaca cgccatgtgg tgaacatgca caccccgctct 180
gagtcgtcca tcgagaattt cctggcacgt tcagcatgcg tgtactacct tgattaccaa 240
acgggagaag ggcccggcga tcagtatttt ggccagtgga ccattaccac gaggagggtt 300
gcgcaattgc gtcgaaagct ggagatgttc acttatctaa gatttgacat ggaaatcaca 360
atcgtgatta ctagttcaca ggatcaatct accatctcga acccagatac accagttttg 420
acgcaccaaa ttatgtatgt accaccagga ggaccaatcc cagcaaaagt cgatgattac 480
agttggcaaa catccacgaa tcccagcgta ttctggactg aagggaatgc gcctgcccgr 540
atatccatcc cattcattag cgttggaaat gcatacagta gcttttatga cgggtggtcg 600
aacttctcac aaaacgggcg gtatggctac aataccctca acaacatggg acaattgttc 660
tttaggcacg ttaacaaacc cagccctaat actgtcacaa gcgtcgcccg catatacttc 720
aagcctaagc acgtgagagc ttggatcccg cgaccaccgc ggttgtgtcc atacataaat 780
gcgggagacg tgaacttcac tccgacacca gtgactgaaa agcgaaagga cctaataacc 840
acg 843

<210> 48

<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E4, strain DuToit

<400> 48
ggagatgtgc aggacgcagt ggctggggcc atagtgcgtg tggctaatac tctcccatca 60
ggcccctcaa acaatgaggc tatacccaac ttaacagccg tagaaactgg acacacctcg 120
caggtgacac cgggtgatac aatgcagacg cgccacgtag tgaacatgca cactcgttct 180
gagtcgtcaa tcgagaactt cctggcgcgg tcagcatgtg tatactacct cgattaccga 240
acaggaacgg ggcctggcaa tcaatacttt agccagtgga ctattaccac aagacgagtt 300
gcgcagctgc gtcgaaaatt ggagatgttc acctatctaa ggttcgacat ggagatcacg 360
attgtaataa cgagttcaca agatcagcct accgtccgaa acccagacac accggtcttg 420
acacaccaaa tcatgtatgt gccaccagga gggccaatcc cagcaaaggt cgacgattac 480
tgttggcaaa catccacaaa ccccagtgtc ttctggactg aagggaacgc accagcccgg 540
atatccatcc cgttcatcag tgtcgggaat gcatatagta gtttctacga tggatggtca 600
aatttctcgc aaaatgggcg gtatggctac aacaccctga acaacatggg gcaattgttt 660
ttcaggcatg tcaataaacc cagtcccaac actgtcacaa gtgttgcccg catatacttc 720
aagcccaaac acgtgaaggc atgggtcccg cgaccaccgc gattgtgccc ttacattaat 780
gctggagatg taaatttcac ccccacatcg gtcactgaga agcgagcgag cctgataacc 840
aca 843

<210> 49
<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E4, strain Shropshire

<400> 49
ggggacgtgc aagatgccgt gactggagcc atagtgcgtg tcgccgacac actgcacacg 60
ggaccctcga acaacgaagc aatacccaat ttgacggccg tggaaacagg gcatacatcg 120
caagtgacac caggcgatac aatgcagacg cgtcacgtgg tcaacatgca cacccgttca 180
gagtcatcaa ttgagaactt cctagctcga tctgcgtgtg tgtattacct cgactatcaa 240
acagggtcag gacctggcac ccaatacttc ggccagtgga ccatctccac aaggagagtt 300
gcgcaactgc gccggaagtt ggaaatgttc acctacctaa gatttgacat ggaaataaca 360
atcgtgatca ccagttcgca agatcactcc accatctcaa atccagatac accaatcatg 420
acgcaccaaa ttatgtacgt accaccaggg ggtccaatcc cggcgaaggt cgacgactat 480
agctggcaaa catctacaaa ccctagtgta ttttggacag aagggaacgc acccgcccgc 540
atatccattc cattcattag tgtcggaaat gcctatagca gcttctacga cgggtggtca 600
aatttctcgc aaaacggccg atatggatac aacactttga acaacatggg acaactattc 660
ttcagacacg tgaataagcc cagccccaac accttcacaa gtgttgcccg tgtatacttc 720
aagccaaaac acgtgaaggc gtggattcca cgaccaccgc gattatgtcc atacataaat 780
gcgggagacg tgaatttcaa accaacaccc gtgaccgaaa agagggcgag cttaatcacc 840
aca 843

<210> 50
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E5, strain Noyce

<400> 50

```
ggagactcag agcacgcagt ggaaagcgcc gtatctaggg tggcagatac aattatgagt     60
ggcccgtcaa actcccaaca ggtccccgct cttactgcag ttgaaactgg acacacatcg    120
caagttgttc caagtgatac catccaaacc agacatgtgc agaatttcca ctctaggtcc    180
gagtcgacca ttgaaaattt cctgagtagg tcagcatgtg tgcatatcgc caattacaac    240
gcgaagggcg ataagacgga tgtggacagg tttgacaggt gggagatcaa cattcgtgaa    300
atggtgcaac tacgtaaaaa gtgtgagatg ttcacatatc tacgctatga tattgaagtt    360
acatttgtta taaccagcaa acaggatcag ggccccaaac taaaccagga tatgcctgtt    420
cttacccacc aaattatgta cgtacccccca ggaggttcag tacctagcac cgttgagagc    480
tatgcgtggc aaacatcaac aaaccctagc gtgttttgga ccgaggggaa cgctccagct    540
agaatgtcca taccctttat cagcataggg aacgcttata gtagcttcta tgatggatgg    600
tcacacttta ctcaaaaagg ggtctacgga tacaacacat taaacaagat ggggcagcta    660
tttgtcagac atgtgaacaa acagaccccc acgccagtta ctagtaccat aagggtttac    720
ttcaaaccaa agcacattag agcttgggtc cctaggcccc cgcggttatg cccctatgtg    780
aacaagacaa atgtaaactt catcaccaca caggtaacag aacctacaaa tgacctcaat    840
gacgtgccca agtctgagca taacatgcac acatat                               876
```

<210> 51
<211> 867
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E6, strain D'Amori

<400> 51

```
aacgacgttc agaacgcggt ggaacggtca attgttcgtg tagcggacac attacccagt     60
gggccaagca actcagaaag cataccagca ctcacagcag ccgagactgg acatacctcg    120
caggtcgtcc ccagcgacac catccagacg cgacatgtga ggaattttca cgttcggtct    180
gagtcatcgg tagagaattt tcttagcagg tcagcttgcg tgtacatcgt ggagtacaaa    240
acccgggaca cgactcccga caagatgtat gatagctgga ttatcaatac caaacaagtg    300
gcgcagttga gaaggaagct ggagttcttt acctatgtca gattcgacgt ggaagttacc    360
tttgtcataa ccagcgtgca agatgactcc acaaaacgga acaccgacac cccagtgcta    420
actcatcaaa ttatgtatgt gccgccagga gggcccatac cacaagcggt ggacgattat    480
aactggcaaa cttccaccaa ccccagcgta ttttggactg aggggaacgc gccaccaagg    540
atgtctattc cgttcatgag tgttggcaat gcatacagta acttctacga cgggtggtcc    600
cacttttctc aaactggggt ttacgggttt aacaccctaa acaacatggg taagttatat    660
ttcaggcatg taaacgacag gactattagc ccaatcaaaa gtaaggtcag aatatatttc    720
aaacccaaac acgtgaaggc atgggtaccc agaccgccga gattgtgtga atacacccac    780
aaggataacg tggactatga accaaagggg gtcacaacat cacgcacttc aatcaccatc    840
accaactcca cacacatgga gacgcac                                         867
```

<210> 52
<211> 867
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E6, strain Cox

<400> 52

```
aatgacgttc aaaatgcagt cgagcaatca attgttcgtg tggctgacac gttacccagt     60
ggacccagta attcagagag cataccggca ctgacggccg ccgagactgg ccatacttct    120
caagttgtgc ccagtgatac tatacagaca cgccacgtaa aaaactttca tgtgaggtcg    180
gagtcgtcag tagagaactt tctcagtagg tccgcttgcg tgtatatagt gggatacaag    240
accacagatg cgacccctga caaaatgtat gacagctggg ttatcaacac aaggcaggtg    300
gcgcagctaa ggagaaaatt agagttcttc acctatgtta ggtttgatgt tgaggtcacc    360
tttgtgataa caagcgtgca agacgattca actagacgga acacagacac cccgttcta     420
```

acccaccaaa tcatgtacgt acccccaggt gggcccatcc cgcaggcagt ggacgactac 480
aattggcaaa cttccacaaa tcccagtgta ttttggacag aagggaatgc cccaccaaga 540
atgtccatac cattcatgag cgtaggtaac gcatacagca atttctatga tgggtggtct 600
cacttctctc aaactggggt gtacggtttc aacaccctga acaacatggg caagctatac 660
ttcaggcatg tgaacggcaa gacaataagc cctatcgcaa gcaaggttag gatttacttc 720
aaaccaaagc atgtgaaggc atgggtgccc agaccaccgc gattgtgtga atacacccac 780
aaggacaatg tggattacga accaaaggga gtcacaacat cccgtacatc tatcacaatt 840
agcaattcca ctcatatgga aacatat 867

<210> 53
<211> 867
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E6, strain Burgess

<400> 53
aacgacgttc agaacgcggt ggaacggtca attgttcgtg tagcggacac attacccagt 60
gggccaagca actcagaaag cataccagca ctcacagcag ctgagactgg acatacctcg 120
caggtcgtcc ccagcgacac catccagacg cgacatgtga agaattttca cgttcggtct 180
gagtcatcgg tagagaattt tcttagcagg tcagcttgcg tgtacatcgt ggagtacaaa 240
acccatgaca cgactcccga cgagatgtat gatagctgga ttatcaatac cagacaagtg 300
gcgcagttga gaaggaagct ggagttcttt acctatgtca gattcgacgt ggaagttacc 360
tttgtcataa ccagcgtgca agatgactcc acaagacaga acaccgacac cccagtgcta 420
actcatcaaa ttatgtatgt gccgccagga gggcccatac cacaagcggt ggacgattat 480
aactggcaaa cttccaccaa ccccagcgta ttttggactg aggggaacgc gccaccaagg 540
atgtctattc cgttcctgag tgttggcaat gcatacagca acttctacga cgggtggtcc 600
cacttttctc aaactggggt ttacgggttt aacaccctaa acaacatggg taagttatat 660
ttcaggcatg taaacgacag gactattagc ccaatcacaa gcaaggtcag aatatatttc 720
aaacccaaac acgtgaaggc atgggtaccc agaccgccga gattgtgtga gtacacccac 780
aaggataacg tggactatga accaaagggg gtcacaacat cacgcacttc aatcaccatc 840
accaactcca cacacatgga gacgcac 867

<210> 54
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E7, strain Wallace

<400> 54
ggcgacaccg aaacggctat tgacaatgca atcgccaggg tagcagatac ggtggcgagc 60
ggtcctagta attcgaccag tatcccagca ctcacagcag ttgagacagg tcacacgtca 120
caagtcgagc ccagcgatac agtgcaaact agacatgtca aaaactacca ctcgcgttct 180
gagtcaaccg tggaaaactt tctaagtcgc tccgcttgtg tgtacatcga agagtactac 240
accaaggacc aagacaatgt taataggtac atgtcgtgga caataaatgc cagaagaatg 300
gtgcaattga ggagaaagtt tgagctgttt acatacatga gatttgatat ggaaatcacg 360
tttgtaatca caagtagaca actacctggg actagcatag cacaagatat gccgccactc 420
acccaccaga tcatgtacat accaccaggt ggcccggtac caaacagcgt aacagatttt 480
gcgtggcaga catcaacaaa ccccagtatt ttctggacag aaggaaacgc gccacctcgc 540
atgtctattc cattcatcag tattggcaat gcatatagca acttctatga cgggtggtca 600
cacttttccc aaaacggtgt gtacggatac aacgccctga acaacatggg caagctgtac 660
gcacgtcatg ttaacaagga cacaccatac cagatgtcaa gcacaatccg agtgtatttc 720
aaacccaagc acatccgagt atgggtccca cggccgcctc gactgagccc gtacatcaaa 780
tcaagtaatg taaattttaa ccccacgaac ctgacggacg agcggtcatc catcacatat 840
gtgcccgaca ctatacgtcc agatgtgcgc accaac 876

<210> 55
<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E8, strain Bryson

<400> 55
```
ggtgatgtcc agaatgcagt tgagggggca atggttagag ttgcagatac cgtgagcact     60
agcgccacca actccgaaca agtgccgaac ctgaccgcgg tggagaccgg tcacacatcg    120
caggtagtgc ccggcgacac tatgcagacc aggcacgtag tgaacaagca tgtgcgatct    180
gaatctacaa ttgaaaattt cctcgcacgt tcagcctgtg tgtactttct tgagtacaag    240
actggtacca agactgactc caacgccttc agcaattggg tcatcacaac gcgcaaggtt    300
gcgcagctga ggcgcaagtt ggagatgttt acatacttaa ggtttgatat ggagattact    360
gtggtcatta ctagctccca agaccagtcc acatcacaaa atcaaaatgc gcccgtcctg    420
actcaccaga ttatgtatgt accacctggt ggcccagtgc ccactagcgt tgatgattat    480
tgctggcaaa catccacaaa cccaagcata ttttggacgg aaggaaacgc acctgccaga    540
atgtccatcc cctttatcag cattggaaat gcttatagca acttttatga tgggtggtca    600
catttctcac agaacggagt ctatggtttt accaccttaa acaacatggg ccagctgttt    660
tttaggcatg ttaacaagcc taacccggcg acaataacca gtgtggcccg catttacttc    720
aagccaaaac atgtgagggc ctgggtgcct agaccgccac ggttgtgccc ttacatcaac    780
agtagcaacg tgaacttcga cccaaaacct gtggcagagg tcaggtctag catcatcacc    840
acc                                                                  843
```

<210> 56
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E11, strain Silva

<400> 56
```
ggtgatgtgg ttgaagccat tgagggcgca gttgctagag tagcagacac tatcagcagc     60
ggcccaacaa attctcaagc agtcccagca ctcacagcgg tggagactgg acacacctcg    120
caagttgtac caggtgatac catgcagacc agacacgtaa agaattacca ctcacgatca    180
gaatcgacca ttgaaaattt tctgagtagg gcggcttgtg tctacatggg tgagtattac    240
actacaaata cagatgagac caagagattt gctaattgga caatcagcgc aaggcgcatg    300
gtacaaatga ggaggaagct tgaaatgttc acgtacgtcc gtttcgacgt ggaggtgaca    360
ttcgtaatta ccagcaaaca ggaccaaggg aatcggttgg gacaagatat gccccegctc    420
acacaccaga taatgtacat cccgccaggt ggtcgtatac ccaaatccac cacagattac    480
gcatggcaaa cgtcgacaaa ccccagcatc ttttggacgg agggtaacgc gccccccagg    540
atgtccattc ctttcatgag cattggaaac gcatatagca atttttatga cggttggtct    600
cacttctctc aaaatggcgt gtacggatat aacacactaa accacatggg tcaattatac    660
atgcgccatg taaatggacg atcacctctt ccaatgacca gcacggtgag ggtgtacttc    720
aaacccaaac atgtgaaaac atgggtgcca cgacccccaa gattgtgcca atacaaaaac    780
gcctcgacag taaacttttc acccacaaac atcacagaca agagggatag catcacttac    840
attccagaca ccgtgaaacc cgacatgaca acatat                              876
```

<210> 57
<211> 861
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E13, strain Del Carmen

<400> 57
ggggatgaga gtgcaaaggc tacagtttcc aacacacagc ctagcggtcc aagtaattct 60
gtcagcgtgc caatgcttac tgctgctgag accgggcaca catctcaagc agtacccagt 120
gacactatac agaccaggtg cgtagtgaac caacacaagc ggtcggaatc atccgtggaa 180
aatttcctgt gtcgctccgc ttgcgtatac tacacaacct atgacactca cggggatgca 240
gccgacgcaa agtacgccag ttggacgata accacccgaa aagctgcaca gctgcggaga 300
aaactagaga tgttcacata cttgaggttt gatttagaag tgacattcgt tataacaagt 360
gcacaagtaa catctaccaa taaacgtcag gacacgcctg ttctcacgca tcaagtcatg 420
tacgtgccac caggtggtgc agtacccgct agtgtggacg attatgcgtg gcagacgtcc 480
acaaacccaa gtatcttctg gacggaaggg aatgcaccag cacgcatgtc tatacccttt 540
atcagcgtgg gcaacgcata cagtagcttc tatgatgggt ggtccaactt tacacagaat 600
ggagtttacg ggttcaacac gctaaacaac atgggaaagc tatacgtacg acacgtcaat 660
ggagctagcc ccggccctgt gaagagtacc atacggtttt acatgaagcc caaacacgtg 720
aaggcttgga tacccagacc tcctcgcctc tgcgagtacg aaaaatcagg caatgtaaac 780
ttcaaaccca agggcgtgac agagagccgg acgtctatca aattagaaaa accaaacccct 840
gcgtccaaat taatgaacca c 861

<210> 58
<211> 894
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E14, strain Tow

<400> 58
aatgatccag agcaagctat aaatcgggcg ctagcgaggg tggcagacac agttcgtagt 60
gggccgtcta actctgaaca aattcccgca ctgacagccg tggagacagg gcatacatca 120
caagtcgtcc ccagtgacac aatgcaaacc cggcatgtga agaattacca ctccaggtca 180
gagtcaacaa tagagaactt tttgtgtaga tcggcttgcg tgcacatcgc aacatacaag 240
gctaaaggcg gagctggaga cgtcgaccgg tacgacagct gggacataaa cataaaagag 300
ctggtacagt tgcgacgcaa gtgcgagatg tttacgtacc taaggtttga tatggaggtc 360
acctttgtga ttaccagcat acaggagcag ggcaaagcac tgacccagga catgccggtg 420
ctaacgcacc aaataatgta cgttccaccg ggcggtgccg tgcctagtgg tgcagaaagc 480
tttgcgtggc agtcatcaac gaatcccagt gtgttctgga cagaaggcaa tgcaccagca 540
cgtatgtcta taccctttat aagtattggg aacgcttaca gtaatttcta tgatgggtgg 600
tcccacttta cccagaacgg tggttacggg tacaacacac taaacaaact gggtaagatc 660
tacgtcaggc atgtgaacaa acaaaccccc acggatgtca ccagcaccgt gcgaatttac 720
ttcaagccca aacacgtgcg agcttgggtg cctcgccccgc ctagactatg tccttataag 780
aacaaggcaa atgtaaactt tgaagttact agtgtaacca ctgccagaac gagtcttaat 840
gatgtcccca ctcccaacca cagtagtagc gtgcacctgc gcatgcacac gcac 894

<210> 59
<211> 882
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E15, strain CH96-51

<400> 59
ggtgatgacc aacacaagac caatacagtg acagacacag agcagagtgg cccgtcaaat 60
tccgaacgcg tcccagccct cacagcagtg gagactggcc acacttcgca ggtcgtaccc 120
agcgacacag tgcaaactcg ccacgtacgc aattaccact caaggacaga gtctacctta 180
gagaattttc ttggtaggtc agcatgtgtg cacatcgaca catacaaggc taagggtgaa 240
aaaggatctt ctgagaggta cgcgtcatgg gagataacta acagggagat ggtgcaattg 300
cgccgaaaat gtgagatgtt cacatatatg aggtatgacg tggaaataac atttgtgata 360
accagctacc aggagcaggg cacacgattg gcccaggaca tgcctgtact aacacaccaa 420
atcatgtacg tgcccccggg tgggcctgtg ccaacaagca cggagagcta tgcatggcag 480

```
acctcaacga accctagcgt cttttggact gagggcaacg caccaccgcg tatttccata      540
cccttcatca gcataggaaa tgcgtactgc aacttttatg atgggtggtc acatttctca      600
caagatgggt cctatggcta cacagcgctc aatagaatgg ggaaaatata tattagacat      660
gtaaataagg agacccccac acaggtcatt agtaccgtga ggatgtacat gaaaccaaaa      720
cacattcgcg catgggtgcc cagacccccc cggctgtgca aatacctaca ctcaggcaac      780
atgaacttca acgtggagga cattacagag gagcggaacg atataaacca tgtacccacc      840
cccagccaca gcagtagtgt gcgtgtgcgt cttggcacca ca                         882

      <210> 60
      <211> 867
      <212> DNA
      <213> Enterovirus

      <220>
      <221> misc_feature
      <222> (0)...(0)
      <223> E17, strain CHHE-29

      <400> 60
ggtgatgttg aggactcagt aaacagagca gtggttaggg tagcagacac catgccaagt       60
ggaccatcca attcgcaggc agtacctgcc ttgacagccg ctgagacagg tcacacgtct      120
caagtggtgc ctggtgataa catccaaaca cgtcatgtgc acaactacca ctccagaact      180
gaatccagta tcgaaaattt cttcgggcgt tccgcatgtg tagtggtcaa aacatataaa      240
atgggtcaaa aagttgtagc tacagacaga tatgatagtt ggatgatttc cattagggac      300
atggtacaac taagacggaa gtgtgaaatg ttcacgtaca tgagatttga tttagagatc      360
accttcgtgg tcacgagtta ccaacaatat agtacatcct tgacacagga catgccagtg      420
atcacgcatc agttcatgta tgtgccgcct ggggggtccgg ttcctgagag tgtaaatagc     480
tacgcttggc aaacgtcaac caatcccagt atattctgga ctgagggtaa tgccccagca      540
aggatgtcca ttcccttcat cagtgttggg aacgcatata gctgcttcta cgatggctgg      600
tcacacttca cacagaaggg ggtttatggt tataacactc tcaacaacat gggcaaattg      660
tacatgcgac acgtgaacaa aaatagcccc acagagatca taagcactct tcgtgtgtat      720
ttcaagccaa agcacgtgaa agcgtgggta cccagaccac ccaggctatg tccatacaaa      780
tataaggcaa atgttgactt tgaagtgact ccaatcacag acaagcgaga ctccataacc      840
agcataccag tccccaagca cactcat                                          867

      <210> 61
      <211> 861
      <212> DNA
      <213> Enterovirus

      <220>
      <221> misc_feature
      <222> (0)...(0)
      <223> E18, strain Metcalf

      <400> 61
ggggataacc aggatcggac ggtcgccaac acacagccta gcggtccgtc caactccacg       60
gaaattccag ccttaacagc ggtggaaacg gggcacacct cacaagtgga tcccagtgac      120
actatccaga ccaggcacgt ggtaaacttc cactcacgtt ctgagtccac tatagaaaat      180
ttcatggggc gtgcagcatg tgtgttcatg gatcagtata aaatcaatgg agaagagacg      240
tccactgata ggttcgcagt gtggaccata aacataaggg agatggccca attaagaagg      300
aagtgtgaaa tgttcacgta catgcgtttt gatatcgaga tgacaatggt cattaccagc      360
tgtcaagacc agggaacgat actagatcag gacatgcctg ttttgacgca tcaaattatg      420
tacgtcccac caggggggccc aatcccagcc aaagtagata gttacgagtg gcagacatca     480
acaaacccca gcgtcttctg gacggaaggt aatgcaccac cgcgtatgtc tattccattc      540
attagcgtcg gcaatgctta tagctcattt tacgatggtt ggtcacactt cacacaggac      600
ggtacctatg ggtatacaac ccttaatgca atggggaaac tgtacattag gcatgtgaat      660
aggagcagcc ctcatcagat aaccagcacg atcagagtat acttcaaacc caaacacatc      720
aaggcatggg tgccccgacc accacgattg tgcccgtata taaacaaaag ggacgtaaac      780
tttgtagtca cggagataac agactcaagg acttccatca ctgatacacc acacccagaa      840
catagtgtcc tggcaacgca t                                                861

      <210> 62
```

<211> 879
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E19, strain Burke

<400> 62
```
ggagacatcg tggaggctgt ggagggagcc atctcgcgag tggcagatac tgttagtagt     60
gggcccagta actctcaagc agtaccagcc ctcacagcag tcgaaacggg tcacacttct    120
caagtcaatc ctagtgacac catgcagacc agacacgtga caaattacca ctcgcggtca    180
gaatccagca tagaaaattt ccttagccgc tctgcttgtg tgtatatggg cgaatacagc    240
acacaagcat cagatgagac caaaaagtac atgtcatgga ccataagccc aaggaggatg    300
gttcaaatgc gcaggaagtt tgagctcttc acttacctgc gttttgatgt ggagattact    360
tttgtaatca ccagcagaca agtcaaggta gggacacaat taggccaaga tgcccccccg    420
ctaactcacc aagtcatgta tatacccca ggaggcccag tacctgattc agttggtgat    480
tacgcatggc agacttccac taaccctagt atcttttgga ccgaaggtaa tgcatcaccc    540
aggatgtcaa taccсttcat tagcataggt aacgcctata gcaactttta tgacgggtgg    600
tcgcattttc accagaatgg cgtctatgga tacaacacgc tgaaccatat ggggcaactg    660
tacgtgcggc atgttaacgg cccttcacca ttaccagtga caagcacagt cagggtctac    720
tttaaaccca aacacgtgaa ggcttgggta ccgagggcac ccaggctatg tcaatatgta    780
aatgcatcca ctgtgaactt cgagccaaca gacatcactg agtcacgcac tgacatcaac    840
catgttccag acaccgtgaa gccagatctc caaacatac                           879
```

<210> 63
<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E20, strain JV-1

<400> 63
```
ggggacgtgc acgatgcggt ggttggggcc atgacccgtg ttgcagacac gataagtagt     60
gggccaagca attcagaaag cgtgccagca ttgactgcag ccgagacagg acacacatca    120
caggtagtac cgagtgatac catgcagacc agacatgtgc ggaatttcca cacaagatca    180
gagtcttcaa tagaaaattt catgagtcgc tccgcctgtg tctactatac taagtataag    240
accaaagacc cggacccaac ggagatgtac tctagttgga aggttaccac caggcaagtg    300
gcacaactca ggaggaagat ggagatgttc acttatttgc gctttgacgt agaagtgaca    360
tttgtaataa ctagctcgca agatcagtcc acgagtgttg cacaggacgc acctgttctc    420
actcaccaaa tcatgtacat cccacccgga ggcccggttc ccaaatcagg tagggattac    480
tcatggcaat cctgtactaa cccaagtgtt ttctggactg agggtaatgc accaccacgc    540
atgtgtattc cgttcattag tattggaggg gcatatagtt cattctatga cgggtggtcc    600
cactttaacc aacaaggtcc gtacgggtat aacactctca atgacatggg tcaactgtat    660
tttaggcatg tgaacgaggg tagcccaggg gcggtaacaa gctacatcag aatatacttc    720
aaacctaaac atattagagc atgggtgccc agaccaccta gattgtgtca gtatgagaaa    780
caagggagcg ttgacttcaa ggtgcaggga gtaactgatg ctcgtacctc gctcaccact    840
aca                                                                  843
```

<210> 64
<211> 885
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E21, strain Farina

<400> 64
aatgacccag cacaagccgt gttgagtgcg atcggtcgtg tcgctgacac cgtcgctagc 60
gggccatcga attcagagag agttccagtt ctaaccgctg cggagacagg tcatacctca 120
caggtggttc ccagcgatac cattcagacg cgccacgtcg tcaacttcca cacaagatcg 180
gagtcaacaa ttgaaaattt tatgtgtcgc tccgcctgcg tgtacatcgc ccggtacggt 240
actgaaaagc aaggggaaca aatatccaga tacaccaagt ggaagatcac cactaggcag 300
gtggcgcaac tgcgcaggaa gatggagatg ttcacataca tgcgatttga tttggaaatg 360
acatttgtaa tcacaagctc ccagcgtatg tcaacggcat atgattcaga cacaccagcc 420
ctcacccacc aaataatgta cgtgccacct gggggcccgg agccccgtca ttatgaggat 480
ttcgcctggc agacatccac aaatccaagc atattttgga ccgaaggtaa cgcaccacca 540
cgcttatcaa tcccatttat gagtgtggga aatgcctatt gcaattttta tgatgggtgg 600
tctcactttt cacaaagtgg agtgtatggg tttaccacct taaataacat gggacaactg 660
ttcatgcgcc atgtcaataa gtcaacagcg caccccattg atagtgtggt gcgagtttat 720
tttaaaccaa agcatgttaa ggcgtgggtt ccaagacctc cccggttgtg cccatacatc 780
tatgcaagga acgtggattt tgagccacaa ggtgtcactg aatcaagaga aaagataaca 840
ctagataggg atactcacac ccctatgcgc acatgcgggc cgttc 885

<210> 65
<211> 882
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E24, strain De Camp

<400> 65
ggagatgtct gtgaggaagt agagagggct attgtcaggg ttgcagatac tgtcggacgc 60
ggtcctgcta acactgagag tgtaccagcg ctgactgcag ttgaaactgg acacacttca 120
caagttgtac ccggggacac catgcaaacc agacatgtta aaactttca cacgcggtca 180
gaatcatctg tggaaaattt catgtgcaga gcagcgtgtg tgtattatgt ggattaccac 240
acacaaaatg acagtgagga tgaaaaatat gcatcttgga ttatcaacac gagacaggta 300
gcacagctac gcaggaaaat tgagctgttc acatacacta ggtttgatgt cgaaatcaca 360
ttcgtgatca ccaccacaca gcagcaatcc acagctccca accccgacac tcctctgctg 420
acacaccaaa tcatgtatgt gcccccgggt ggcccagtgc caaatagtgc taccgattat 480
tgttggcaat catccacaaa tcccagtata ttctggaccg agggtagcgc accacccaaa 540
atgtcaatac cctttataag tgtgggaaat gcatacagca gtttttatga tgggtggtca 600
catttcactc aaaacggggt gtacgggttc aacactctga acaatatggg caaattatac 660
ttcaggcacg taaatgacaa caccgtaggg ccatatgtga gcaaagcccg catttatttc 720
aaaccaaagc atgtgcgtgc gtgggttccc aaacctccca ggctctgtga atacaacaat 780
cgagccaacg tgaactttga accacgaggg gttaccgatg ccaggtctag tatcacggcc 840
acaaccgaca cgatcactga gagcacaggg atgcaaacga ct 882

<210> 66
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E25, strain JV-4

<400> 66
aatgatccag caactgccat agttagatcg gttgagagag tggctgatac catagcaagt 60
ggacccacta actcagagag agtgccagca ctaaccgccg ttgaaacagg tcacacctca 120
caggtagtcc cgagcgacac catgcaaact aggcatgttg tgaaccatca cattagatca 180
gagtcctcta ttgaaaactt cctgagcagg tccgcctgcg tgtacatcga catgtatggg 240
acaaaagaga atggtgacat caagcgcttc accaactgga gaataaacac acgtcaggtc 300
gtgcagctaa ggcgcaagct ggaaatgttt acatacatta gatttgatgt tgaaatcact 360
tttgtaatca ctagcacaca gggaacaccg actcaaaaga acaaggatac cccagttctt 420
acacaccaaa tcatgtatgt gccaccaggg ggcccaatcc ctgtatctta tgaagattat 480

```
tcttggcaga cctctacaaa tcctagtgtt ttctggacag aagggaatgc cccagcccgt      540
atgtcaattc ccttcatgag cgtagggaac gcctattgta acttttacga cgggtggtca      600
cacttctcac aatcgggtgt gtatgggttc actacactca ataacatggg tcagttgtac      660
tttcgacacg tgaacaagga cacccttgga ccatacaata gcacggttcg ggtttacttc      720
aaacccaaac atgtgaaggc atgggtaccc agaccaccgc gcctgtgcga ctacgtttac      780
gcacataatg ttgacttcac accaaaaggg gttactgaca gcagggacaa gatcaccctg      840
gaccgtgatg aacacgtgcc gtcagtggtt aaccac                                 876
```

<210> 67
<211> 870
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E26, strain Coronel

<400> 67

```
ggagatgatc caccgcattc gatctcaaac acggttgcaa acaccaaccc tagtggtcca       60
accaactcag aaaggatccc agcgctcaca gcagcggaaa ctggtcacac ctcgcaggtg      120
gtcccgagtg ataccgtaca aactcgttgt gtgaaaaact tccacactcg atcggagtca      180
tcaattgaga actttttgtg cagatcagct tgcgcacaca tgtcatcgta tgaggccttc      240
ccaacaacaa cacaagacgg tacacaaagg ttcgccaatt ggacgattag tgtgaaagac      300
atggtgcagt tgaggaggaa atgtgagatg ttcacgtact taagatttga catggaggtg      360
acttttgtga taactagtgt gatcgaaact acaaaaggga aagtaccggc accagcagtc      420
acacaccaag taatgtacat tccaccaggc ggacctattc cagctagcgt tgaaagttat      480
gcctggcaaa catccaccaa cccaagcgtg ttttggacag aagggaatgc tcccccacgc      540
atgtctatac catttatcgg cattggtaat gcctacagca tgttctatga cggatgggcc      600
agtttcagac aatcgggtgg atatggatac agcaccctga accacatggg ccagatattc      660
gtaagacacg tgaatgcaac cataccaaac ttgatcagca cagtcaggat atatttcaag      720
cccaagcacg ttagggcttg gattcctaga ccgcccaggg tgtgtcagta catttacaag      780
gcaaatgtag actacgcagt gtcaaatatc actgaaaagc gagatagtat aagatggaca      840
ccaacaaccg gtccgtcaat gacatcccac                                       870
```

<210> 68
<211> 855
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E27, strain Bacon

<400> 68

```
ggtgacgacg caaggactgt tagcgacaca caaaagagcc agccatctaa ctctgagcaa       60
gtgcctgcct taacagcggt tgagactgga cacacctctc aagttgagcc cagtgataca      120
gtacagacac gacatgttgt caactcacac agtaggacag agtcgacaat tgagaatttc      180
tttgggaggg ctgcgtgtgt gagggtgaga gagtactcta tagggcatga tttggcagcg      240
gacgaaacat atgatagctg ggccattaca gtgcgagaca tggtgcagct tcgtaggaag      300
tgtgagatgt tcacatacat gaggtttgac ttggaagtga cgctagtcat caccagctat      360
caagaaccag ggacaatcac cacccaggat atgcccgtcc taacccacca gattatgtat      420
gtgccgccag gaggcccggt cccagccaag gctgacagtt acgcgtggca aacgtcaaca      480
aatcccagta tattctggac cgaaggcaac gctccacctc ggatgtctat cccatacatt      540
ggcatcggca atgcatatag cagcttttat gacgggtggt cgagcttcaa caactcgggt      600
gtgtatggct acacaaccct gaataacatg ggtaaactgt acttcagaca cgtgaacaaa      660
cacagcccaa acactattaa gagcactgtg aggatatatt tcaagcccaa gcacgtccag      720
gcgtgggtcc caagaccacc gcgcttgtgc ccgtatctga ataagaggga tgtcaacttt      780
gaagtgcaac ccgttacgag caagagagac agtattaact gggtgccaca aacaaaccgc      840
caagtgtaca atcat                                                        855
```

<210> 69

<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E29, strain JV-10

<400> 69
```
aatgaaccta gtagtgccat tgagagagca attgtgcgcg tagcagatac tatggccagt      60
gggcctgcaa actcagagca aatccctgcc ctaaccgctg ctgagactgg tcacacctcg     120
caagtggttc ccagcgacac tatgcaaacc cgccatgtat gtaactacca caccagatct     180
gaatcatcga tcgagaactt cctatgcagg gctgcatgtg tctacatagt gagttacaaa     240
acacagggcg acgaacaaac cgacaaatac gctagttggg agatcaacac gcggcaggtg     300
gcacagttaa ggagaaaatt ggaattcttt acttacataa gatttgacat ggaggtaaca     360
tttgtgatca ctggttcaca agacaccagc acacagacta acacggatac gccagtgcta     420
acccatcaaa ttatgtatgt gcctcccggt ggtccagtac cgacatcagc cacagattac     480
agctggcaga catctacaaa tcccagtgtg ttctggacag aagggaatgc gcctccccgt     540
atgtccatac ccttcatgag cataggcaat gcgtatgcta atttctatga tgggtggtcg     600
cactttagcc agtcaggggt gtatggttac accacactca ataatatggg taccctgtat     660
ttcaggcacg tgaacaactc gaccatcggg ccttacacca gtgcagttag gatatatttc     720
aagccaaagc acgtcaaagc gtgggtgcca cgaccgccac ggttgtgcga ttacaaacac     780
aaaaagaacg tagactttac tcccacaggt gtgaccacaa ctagagacaa gataaccttg     840
gacaagggga ctcacgtgcc gagcgtatgg aacaca                               876
```

<210> 70
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain Bastianni

<400> 70
```
aatgaccccg aaggtgcact taataaagca gtgggcaggg tagctgatac tatagctagt      60
gggcccgtca atacagagca aattcctgca ttgacagcag tggagacagg gcatacatct     120
caagtggtac ctagtgacac aatgcaaacc cgacacgtgg tcaacttcca tactagatca     180
gagtcatcgt tacagaactt catggggaga gcggcatgtg tatatatcgc ccactatgcc     240
acagaaaagg ctaatgatga tttggacaga tacactaact gggagatcac aactaggcag     300
gtggcacagt tgaggcgcaa gttggagatg tttacgtata tgagatttga cctcgagatt     360
acattcgtaa tcaccagctc ccagcgtact tccaacaggt atgcgtcaga ctcccccccca     420
ttaacacatc aaataatgta cgtgccgccg gggggtccaa ttcccaaggg ttatgaagac     480
tttgcctggc agacgtccac caacccaagt gtgttttgga ccgaaggtaa cgcccctcct     540
aggatgtcaa taccattcat gagcgttggc aacgcatatt gtaactttta tgatggatgg     600
tcccatttca gtcagagcgg tgtgtacggg tacactacat tgaacaacat ggggcgctta     660
tattttagac atgtaaacaa atcaacagga tacccagtaa atagtgtcgc ccgcgtctat     720
ttcaagccca agcatgtgaa ggcatgggta cctcgcgcgc cacgcttatg tccatatttg     780
tatgctaaaa atgtcaactt tgatgtgcaa ggcgtgaccg agtcccgggg taagatcact     840
ctcgaccgtt cgactcacaa ccccgtgtta accact                               876
```

<210> 71
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain Frater

<400> 71
aatgaccctg aaggtgcgct caacaaggcg gtgggcagag tggctgatac aatagccagt 60
gggcccgtca acactgagca aattcccgca ttgacagcag tggaaacagg gcacacatct 120
caagtagtac ctagtgatac aatgcaaact cgacacgtgg tcaacttcca caccagatca 180
gaatcatcgt tggagaactt catgggaaga gcagcgtgtg tgtatatcgc tcattatgct 240
acagagaagg ctaatgatga tttagacaga tacaccaact gggaggtcac aaccaggcag 300
gtagcacagt tgaggcgtaa actggagatg ttcacgtaca tgaggtttga cctcgagatc 360
acatttgtaa tcaccagctc ccagcgcact tcaaccaagt atgcgtcaga ttcccccccca 420
ctaacacacc agataatgta tgtaccgccg gggggcccga tccccaaggg ttatgaagat 480
tttgcctggc agacgtccac caacccaagt gtattttgga cggaaggtaa cgcccccccct 540
aggatgtcga taccattcat gagcgttggt aacgcatact gcaactttta cgacggatgg 600
tcccatttca gccagagcgg tgtgtacggg tacactacat tgaacaacat ggggcacttg 660
tatttcagac atgtaaacaa atcaactgca tacccagtta acagtgttgc ccgcgtctac 720
ttcaagccca agcacgtaaa ggcttgggtg cctcgcgcgc cacgcttatg tccatatttg 780
tatgcaaaaa atgtcaattt tgatgtacaa ggtgtgaccg agtctcgggg aaaaatcact 840
cttgatcgat cgactcacaa ccctgtgtca accacg 876

<210> 72
<211> 877
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain Giles

<400> 72
aacgaccccg aacatgcgtt aaacaacgcc attggtagag tggcagatac gatcgccagt 60
gggccggtga actcggaacg catacctgca ctaaccgcag tggagacagg acacacgtct 120
caagtggtgc caagcgacac catgcaaaca aggcacgtag tcaacatgca tacaagatcc 180
gaatccacca tcgaaaattt catgggaagg gctgcttgtg tatacattgc gcaatacgcc 240
actgataagg ccagtgatga tctggacagg tacaccagct gggagatcac tacgagacag 300
gttgcgcaat tgaggagaaa gctggagctg tttacataca tgaggtatga cttagaagtt 360
acctttgtca ttaccagttc ccagcgcact tcgactacat atgcatcaga ctccccgcca 420
ttgacccacc aaattatgta tgtgcctccc gggggcccta ttcccatagg acacgaagac 480
ttcgcctggc agacttcaac aaaccccagt gtcttttgga ctgaaggaaa tgccccacca 540
cgtatgtcca taccattcat gagtgtgggc aatgcctact gcaattttta cgatgggtgg 600
tcacatttta accagagtgg ggtgtatgga tacactacac taaacaacat gggtcgctta 660
tatttcaggc atgtaaacag atctactgcc tacccagtta atagtgttgc acgtgtttac 720
tttaaaccca aacacgtcaa agcctgggtc ccacgagcac cacgattgtg cccatacttg 780
tatgctaaga acgtgaactt taatgtgcaa ggtgtgactg actcccgaga caagataacc 840
gtagaccgaa ccaaccatgt acgtatgcgc accacag 877

<210> 73
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E30, strain PR-17

<400> 73
aacgaccccg aacacgtgtt aaacaatgcc gttggcagag tggcagatac aatcgccagc 60
gggccggtga actcggaacg cgtacctgca ctaactgcag tggagacagg gcatacgtct 120
caagtggtgc caagcgatac tatgcaaaca agacacgtag tcaacatgca cacaagatct 180
gaatccacta tcgaaaattt catgggaagg gctgcttgtg tatacatcgc acaatacgct 240
actgacaaag ccagtgacga tttggatagg tacaccagct gggaaatcac cacgagacag 300
gttgcgcaat tgaggagaaa gttggaaatg ttcacataca tgaggtatga cctggaagtc 360
acctttgtta tcaccagttc ccagcgcacc tcgactacat atgcatcaga ttccccacca 420
ttgactcatc agatcatgta cgtgcctccc gggggcccca ttcctatagg atacgaggac 480

```
ttcgcctggc aaacatcgac taaccctagt gtcttttgga ctgaaggaaa tgccccacca     540
cgcatgtcca ttccatttat gagtgtgggc aatgcctact gcaattttta cgatgggtgg     600
tcacacttta gccagagtgg ggtgtacgga tacactacac taaataatat gggtcgtctg     660
tatttcaggc atgtaaacaa atctactgcg tacccggtta atagtgttgc acgtatttac     720
ttcaaaccca aacatgttaa agcctgggtc ccgcgagcac cacgactgtg cccatatttg     780
tatgcaagga acgtgaactt taatgtgcaa ggtgtgactg actcccgaga aaagataacc     840
atagaccgaa ccaaccatgt gcccatgcgt aacaca                               876
```

<210> 74
<211> 876
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E31, strain Caldwell

<400> 74

```
ggggacacgg aacatgcagt tgagtcagct atctccaggg tagcagatac cattagctca      60
ggtcctagta acactgttgc tataccagcg ctcaccgcgg cagaaacggg ccacacatcg     120
caagtcaccc ccagcgacaa tcttcagacg cgccatgtta agaactatca ctcccgctct     180
gagtcaacta ttgaaaactt cctgtgtaaa tccgcgtgtg tgcatattgc gtcatacaac     240
gcatacggtg atgttggatc agacagtaga tatgatagtt gggagatcaa catcagggaa     300
atggtgcagt taaggaggaa gtgcgaaatg ttcacctatc tcagatttga catggaggtg     360
acatttgtca tcactagcaa gcaagatcaa gggacttcgc tatcacaaga catgccagtg     420
ctaacacatc agatcatgta cgtgccgcca ggcggatccg tgcccactag cgtccagagc     480
tacgcatggc aaacatccac caacccgagc gtgttttgga cagagggcaa tgccccctgct     540
agaatgtcca tcccattcat tagcataggg aatgcataca gcagcttcta cgacgggtgg     600
tcacatttca cccaacaagg tggctatggc tataatacac tgaacaagat gggtaagttg     660
tttgtaaggc atgtgaataa agaaacacca acccatgtga cgagcacgat acgtgtatat     720
tttaaaccaa agcatgttag agcgtgggtg ccaaggccac ctagattgtg cccgtacatc     780
aataaagcgg actgtaactt cgctgttaca ccactcacca aacagcggtt aggaatcaac     840
gatgtcccgc ggcccagcca cacattacat actcat                               876
```

<210> 75
<211> 875
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E32, strain PR-10

<400> 75

```
aacgaccccg caaccgctat tgaaggagca gtccggcgag tggcggacac gatccagagc      60
ggaccgagca attcggagcg ggttccagcg ttaacggccg ttgagacagg tcacacagca     120
caggttaccc cgagtgatac aatgcaaact agacatgtac acaacttcca caccagatcg     180
gagtctagca tcgagaactt cctcagtaga gcagcttgtg tgtacatagg gaaatatagt     240
agcaatgcaa caacacaaga tgaacaatac atgtcatgga caattaatac cagacagatg     300
gtgcagctga gacgcaaatt cgaaatgttc acctacctac gcttcgacgt agaagtcact     360
tttataataa catcgcacca agatcaaggg acacagttca accaggatgc gcccgtaatg     420
tgccaccaaa tcatgtatgt gccacctggt ggcccggtgc ctaagagtgt tgatgacttc     480
acatggcaaa cctctactaa ccctagtgtc ttttggtcag aaggcaatgc accaccgaga     540
atgaccattc cattcattag tatagggaac gcctacagca gcttttatga tggctggtca     600
cacttctctc aaaatggggt ttacgggttt aatgcactca ataacatggg taaactgtat     660
gtgagacaag tgaacctaaa agcccctatg ccagtcagca gtacagttag gatctatttc     720
aaacccaagc atatcaaagc ttgggtaccc agaccaccgc gtctatgtaa gtacctgaag     780
tctgggagtg tcaatttga gcccactgat ttgacagaaa aacggaaatc cagaaagtac     840
atcccaaaaa ctttcagacc agatgtgaga accat                                875
```

<210> 76

<211> 843
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E33, strain Toluca-3

<400> 76
```
ggtgatgtgc atgatgcagt tgtgggtgcg atgtcgcgcg tcgctgatac agtagcaagt      60
ggccctgcaa actctgagag cgtgcctgct ctcactgcgg tagaaactgg acacacgtca     120
caggtgacac caagtgatac aatgcagacc agacacgtac acaacttcca cacacggtcc     180
gaatcgtcaa tcgagaactt cttaagccgc tctgcatgtg tctattatgc aacgtacaaa     240
acaacagcca gcagacccga agaccaattc gttaggtggt ccatttcata ccgccaggtg     300
gcccaactgc gcaggaaaat ggaaatgttc acctacctgc gctacgatgt ggaggtcact     360
tttgtgatta caagttctca ggacccatcg accaacgtaa gccaggatgc tcctgtactc     420
acacatcagt taatgtacgt acccccccggg ggtccagtgc ccaaaaattc aagagactat     480
gcatggcaaa catccaccaa cccgagtgtg ttctggaccg aggggaacgc accaccaagg     540
atatccatcc cctttatcag tgtgggcaac gcatacagtt gcttttatga tggatggtcc     600
cactactcac agacgggggt gtatggttac aacaccttaa acgacatggg ccaattattt     660
gtcaggcacg tgaatgaggc aagcccgggt gcggtgtcaa gtgtagttag gatttacttc     720
aaacccaaac atgtgaaggc atgggtcccg agaccaccac ggttgtgcca atatgttaac     780
gcagcaacgg tgaacttcac tcctgaaggg gtcactaagg cacgtactga tctcatgaca     840
aca                                                                   843
```

<210> 77
<211> 915
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> E34, strain DN-19

<400> 77
```
ggaatagaag aaactattga cacagtgatc accaacgctt tacaactgtc tcagcccaaa      60
ccgcagaaac aactcactgc tcaatccacc gcctcatcca gcggagtcaa ttcacaagaa     120
gtgccagcat tgactgctgt ggagacggga gcttctggtc aagccatacc cagcgacgtg     180
attgagacca gacatgtcgt caattacaaa actagatctg aatcaaccct tgagtcattc     240
tttggtagat cagcatgcgt aaccatactg gaagtagaga acttcaatgc cactaccgaa     300
tcggacaaga aaaagcaatt caccacctgg ccaatcacat acaccaacac agtccagttg     360
cgcaggaaat tggaattctt tacatactcc agatttgatc tggaaatgac ttttgtcata     420
actgagaggt accacacaag taatacagga catgctagaa atcaagtgta ccaaataatg     480
tacataccac cgggtgcgcc aaggcccaca gcacgggatg attacacctg gcaaagttca     540
tccaatccat cagtgtttta cacatatggt agcgcgcctc ccagaatgtc tatcccatat     600
gttggcattg ccaatgcata ctcacacttt tatgacgggt ttgcccgagt tcccctgaaa     660
gatgatacaa ctgactccgg tgacactttt tatggattgg tcaccatcaa tgactttgga     720
acattggctg tgagggtggt gaatgagttc aaccctgcaa ggataacatc aaaggtcaga     780
gtttatatga agcccaaaca tgtgaggtgt tggtgtccta ggccaccgcg cgcagtgccc     840
tatcgtggtg aaggggttga tttcaaacaa gattcaatca cgccaataac agcagtcacc     900
aatattaata ccttc                                                      915
```

<210> 78
<211> 936
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> EV68, strain Fermon

<400> 78
tcaaaccact tacatggagc agaggcagcc tatcaggtgg agagtatcat caaaacagca 60
actgatactg tgaagagtga gattaacgcc gaacttggtg tggtccctag tctaaatgca 120
gttgaaactg gtgcaacttc caacactgaa ccagaagaag ccatacaaac tcgcacagta 180
ataaatcagc atggtgtgtc ggagacgtta gtggagaatt ttcttggtag ggcagcccta 240
gtgtcaaaga aaagttttga atacaagaat catgcctcat ccagcgcagg gacacacaaa 300
aacttttta aatggacaat taatactaag tcttttgtcc agttaagaag aaagctggaa 360
ttattcacat accttaggtt tgatgctgaa atcaccatac tcacaactgt ggcagtaaat 420
ggtaataatg acagcacata catgggtctc cctgacttga cactccaagc aatgtttgta 480
ccaactggtg ctcttactcc aaaggagcag gattcatttc attggcaatc aggcagtaat 540
gctagtgtgt tctttaaaat ttctgatccc ccagctagaa tgactatacc ttttatgtgc 600
atcaactcag catattcagt tttttatgat ggctttgctg gatttgagaa aaatggtcta 660
tatggaataa acccagctga cactattggc aacttgtgtg tcagaatagt gaatgaacat 720
caaccagttg gttttacagt gaccgttagg gtttacatga agcctaaaca tataaaagca 780
tgggctccac gaccaccgcg aaccatgcca tacatgagca ttgctaatgc aaattacaaa 840
ggtagagata cagcaccaaa cacacttaat gccataattg gtaatagagc gagtgtcaca 900
actatgcctc acaacatagt aaccaccggt ccgggt 936

<210> 79
<211> 861
<212> DNA
<213> Enterovirus

<220>
<221> misc_feature
<222> (0)...(0)
<223> EV69, strain Toluca-1

<400> 79
aatgaccagc acaatggggc gatcgttgcc aacacaacag ctagcggacc ttctaattcg 60
gaaagcatac cggcacttac tgcggctgag actggccaca catcgcaggt tgtccctagc 120
gacaccatcc agacaagaca tgtgaaaaac taccactcgc gttcagagtc caccatagag 180
aacttcctgt gtagatctgc ctgtgtgtac tacaccacgt acaacactca gggcgagcaa 240
gcacatgata aatacgcaag ttggccaatc acgactagaa aagttgccca actgcgcagg 300
aagctggagt tctttaccta cctgcggttt gatctcgaga tcacgttcgt gatcacgagc 360
gcccagatca catccacgaa ccaaaaccag gatgccccag tactcacaca tcaggtgatg 420
tatgtacccc caggggggt ggtaccgcgc agtgtggatg actatagttg gcagacttcc 480
accaatccca gcatcttctg gacagaaggg aacgcacctc ctcgtatgtc aataccattc 540
attagtgtgg gcaacgccta cagcagcttt tacgacgggt ggtcacactt tgaacaaacc 600
ggggtatatg gattcaatac ccttaataat atggggactt tgtacgccag gcacgttaac 660
ggtgctagtc ccgggccagt caagagcacc attaggatat atatgaaacc taaacatgtg 720
aaagcgtgga tacctaggcc cccacggttg tgcgactatg tgaaatctgg caacgtcaac 780
tttgaaccaa aaggagtcac cgagagcaga ccatctataa agttagaaaa gacctcaagt 840
gggcacaggc tgacaaccca c 861

<210> 80
<211> 7
<212> PRT
<213> Enterovirus

<400> 80
Met Tyr Val Pro Pro Gly Gly
1 5

<210> 81
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa(Position 3) = Val or Ile

<223> Xaa(Position 5) = Pro or Thr

<400> 81
Met Tyr Xaa Pro Xaa Gly Ala
1 5

<210> 82
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa(Position 3) = Gln or His

<400> 82
Phe Gly Xaa Gln Ser Gly Ala
1 5

<210> 83
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 3) = Ala or Val

<400> 83
Thr Ala Xaa Glu Thr Gly His
1 5

<210> 84
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 7) = Ala or Val

<400> 84
Thr Ala Val Glu Thr Gly Xaa
1 5

<210> 85
<211> 7
<212> PRT
<213> Enterovirus

<400> 85
Gln Ala Ala Glu Thr Gly Ala
1 5

<210> 86
<211> 7
<212> PRT
<213> Enterovirus

<220>
<223> Xaa (Position 2) = Phe or Tyr

<223> Xaa (Position 3) = Ile or Val

<223> Xaa (Position 7) = Ala or Gly

<400> 86
Met Xaa Xaa Pro Pro Gly Xaa

1 5

DECLARATION
FOR PATENT APPLICATION

(X) Original () Supplemental () Substitute () PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"TYPING OF HUMAN ENTEROVIRUSES,"** which is described and claimed in the specification

(check one) [] which is attached hereto, or
[] which was filed on , as Application Serial No. and with amendments through (if applicable), or
[X] in International Application No. PCT/US00/07828, filed March 24, 2000, and as amended in accordance with the Preliminary Amendment provided herewith

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO
PCT/US00/07828	PCT	24/03/2000	X	

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

3-00

Full name of third inventor: KILPATRICK, David R.

Inventor's signature: David R Kilpatrick Date: 9/25/01

Residence: 1095 Fulton Court, Norcross, Georgia 30093 GA

Post Office Address: 1095 Fulton Court, Norcross, Georgia 30093

Citizenship: United States of America

4-00

Full name of fourth inventor: PALLANSCH, Mark A.

Inventor's signature: [illegible] Date: 9/25/01

Residence: 4749 Mockernut Court, Lilburn, Georgia 30047 GA

Post Office Address: 4749 Mockernut Court, Lilburn, Georgia 30047

Citizenship: United States of America

APPLICATION NUMBER	FILING DATE
60/127,464	March 31, 1999

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-60

Full name of first inventor: OBERSTE, Steven

Inventor's signature: [signature] Date: 9/25/01

Residence: 5110 Sunset Maple Trail, Lilburn, Georgia 30047 GA

Post Office Address: 5110 Sunset Maple Trail, Lilburn, Georgia 30047

Citizenship: United States of America

9-10

Full name of second inventor: MAHER, Kaija

Inventor's signature: [signature] Date: 09-25-01

Residence: 3014 Silvapine Trail, Atlanta, Georgia 30345 GA

Post Office Address: 3014 Silvapine Trail, Atlanta, Georgia 30345

Citizenship: United States of America

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division

Application deficiencies found during scanning:

☐ Page(s)__________ of ______________________________ were not present for scanning. (Document title)

☐ Page(s)__________ of ______________________________ were not present for scanning. (Document title)

☐ ***Scanned copy is best available.*** FIG. 2 -

IS/are too dark